THE USE OF STICKERS IN LEGUME INOCULATION WITH *Rhizobium*

BY

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Information concerning the advantage of using stickers in legume inoculation with *Rhizobium* is still limited and the reasons why stickers may enhance nodulation are still not clear. The objectives of this study were (1) to investigate enhancement of nodulation and (2) to explain the enhancement effects.

Gum arabic (GA) sticker was used in this study. The enhancement effect of GA on nodulation was studied by comparing the effect of seed pelleting technique and normal inoculation on nodulation of *Trifolium repens* L. (white clover), *Centrosema pubescens* (L.) Benth., and *Calopogonium caeruleum* (Benth.) Hemal. Reasons for the enhancement effects were investigated by counting *Rhizobium* cell numbers stuck onto seed using GA and water as stickers, by studying the effect of increasing GA concentration on *Rhizobium* cell numbers in a defined medium, and by studying the effect of

GA on pectinase and cellulase production by Rhizobium (R) and white clover seedlings (S) grown in a sterile water. Pectinase and cellulase activities were measured as percentage decrease in viscosity (PDV) in 24 h in 0.2 dag pectin L^{-1} and 0.2 dag carboxy methyl cellulose (CMC) L^{-1} solutions, respectively. Experimental design used was a completely randomized block.

A laboratory experiment with white clover showed that nodule number for GA was 1.6 times that for water ($P<0.05$). A greenhouse experiment with Calopogonium caeruleum showed that nodule number for GA was 2 times that for water ($P<0.05$). Gum arabic stuck Rhizobium cells on white clover seed 2.5 to 3.0 times that for water. Increasing GA concentrations in a defined medium increased Rhizobium cell numbers significantly. The relationship between GA concentrations (X) in g L^{-1} and cell numbers mL^{-1}(Y) was $Y = (1.88 + 0.51X - 0.006X^2)10^8$ ($R^2 = 0.88$). Percentage decreases in viscosity in pectin solution for R, R+GA, R+S, and R+S+GA were 10.4, 32.2, 13.2, and 86.4, respectively (SE=9.9), and the PDV's in CMC solution were 1.8, 7.4, 38.7, and 62.9, respectively (SE=10.9).

It was concluded from this study that GA sticker significantly enhanced nodulation. It stuck more Rhizobium cells onto seeds and increased cell numbers. Gum arabic contained pectinase which could "weaken" the plant cell wall and may have induced pectinase and cellulase production by the plants, thus facilitating root infection.

INTRODUCTION

The use of stickers in legume inoculation with *Rhizobium* has been studied by many researchers, including Hastings and Drake (1960), Brockwell (1962), and Waggoner et al. (1979), who found that stickers enhanced nodulation. The reasons why stickers enhance nodulation are still not clear; they may stick more *Rhizobium* cells onto seeds, or act as a source of carbohydrates to increase cell numbers, or increase plant infection by acting as inducers for certain cell wall-hydrolyzing enzymes such as pectinase and cellulase.

The objectives of this study were (1) to verify reports that stickers enhance nodulation and (2) to determine how stickers enhance nodulation.

In this study, gum arabic (GA), a commercially used sticker, was used in conjunction with the legumes *Trifolium repens* L. (white clover), *Centrosema pubescens* (L.) Benth and *Calopogonium caeruleum* (Benth.) Hemal. The latter two species are tropical legumes usually grown as ground cover for rubber plantations in southeast Asia. The experiments were carried out under laboratory, greenhouse, and field conditions. In one of the laboratory experiments, the

effect of GA on nodulation of white clover was also compared with other kinds of commercially used stickers. The GA enhancement of nodulation was studied under laboratory conditions, with the following objectives: (1) to determine the effect of GA concentration on Rhizobium cell number in a defined medium, (2) to study the effect of GA concentration on nodulation of white clover in solution culture, (3) to study the effect of GA concentration on nodulation and growth of white clover in a sandy soil, (4) to study the effect of varying Rhizobium cell number per seed on nodulation of white clover, comparing water and GA as stickers, and (5) to study the effect of GA on pectinase and cellulase production by Rhizobium and white clover seedlings.

LITERATURE REVIEW

Legume Inoculation

Nitrogenous fertilizers are needed for maintaining or increasing crop production. The increase in cost of fuel invariably results in an increased price for N fertilizers. To overcome this problem, research workers have attempted to increase soil N levels through biological processes. The most important process is through symbiotic N_2-fixation by *Rhizobium* in root nodules of legumes. The amount of N_2 fixed by *Rhizobium* with various legumes ranges from 20 to 550 kg N ha^{-1} (Vincent, 1974).

Rhizobium is a weak competitor in soils. Therefore, in order to improve N_2-fixation, inoculation with this microorganism is frequently necessary. This practice is usually highly successful provided that all chemical, physical and biological properties of the habitat are favorable for the survival and multiplication of *Rhizobium*. According to Vincent (1974), legume inoculation is needed where specific rhizobia are absent or sparse or where local rhizobia are ineffective in their N_2-fixing capacity. Allen and Allen (cited by Brockwell, 1982, p. 211) listed the reasons for legume inoculation as follows:

(1) The absence of the same or symbiotically related legume in the immediate past history of the land.

(2) Poor nodulation when the same crop was grown on the land previously.

(3) When the legume follows a non-leguminous crop in a rotation.

(4) When legumes are used for land reclamation.

The history of legume inoculation began with transferring of soil from the vicinity of a good crop to the plant in question. According to Brockwell (1982), within a few years after Hellriegel and Wilfarth had found the relationship between legume root nodules and the assimilation of atmospheric N in 1888, soil transfer of up to 3,000 kg ha^{-1} was a recommended method of legume inoculation.

Currently, the first step in legume inoculation is to isolate Rhizobium strains from a desired host plant. The isolated strains are then grown as pure cultures and tested for their relative abilities to nodulate and fix N_2 on the host plant. The third step is to inoculate legume seeds using the inoculants formulated from the best of rhizobial strains. Success in legume inoculation is influenced by inoculant type, inoculation method, and other factors such Rhizobium strain, legume type, and soil and environmental conditions.

Brockwell (1982) listed four inoculant types: agar cultures, liquid cultures, lyophilized cultures, and

solid-base cultures. Agar or liquid cultures are usually used on a small scale as for laboratory or greenhouse experiments. Lyophilized cultures are not popular with farmers; the mortality rate of this inoculant type on seeds is usually high (Vincent, 1965). McLeod and Roughley (1961) used this inoculant type under field conditions. Of the inoculant types, solid-base cultures are most widely used in practice.

Solid-base cultures are made by placing Rhizobium on a carrier material, such as peat. Other tested carrier materials include lignite (Kandasamy and Prasad, 1971); coir dust or soybean meal (Iswaran, 1972); coal (Dube et al., 1975; Paczkowski and Berryhill, 1979); rotten corncobs (Corby, 1976); mixture of coal, and bentonite and lucerne meal; Ca-sulphate; talc; soil supplemented with lucerne meal; ground straw; soil and ground rock phosphate; and coffee husk compost (Strijdom and Deschodt, 1976). Dommergues et al. (1979) introduced a synthetic carrier material, polyacrylamide gel.

Natural carrier materials such as peat, lignite, coal, and compost are all high in organic matter content with low to high pH. Peat types reported by Roughley and Vincent (1967) had a pH ranging from 7.0 to 8.3. Lignite as reported by Kandasamy and Prasad (1971) contained organic matter ranging from 73.2 to 76.9 dag kg^{-1} with a pH of around 3.8. Coal had an organic matter content of 79 dag

kg^{-1} with physical properties about the same as peat and lignite (Dube et al., 1975).

In peat inoculant production, the peat sample is first dried, powdered, and sieved through a 200-mesh sieve (Roughley and Vincent, 1967). The peat is adjusted to pH 6.5 to 7.0 by mixing with lime ($CaCO_3$), if necessary. The next step is sterilization. There are four methods of peat sterilization: dry heating at 170°C for 1 h (Gunning and Jordan, 1954), steam sterilization (Newbould, 1951), autoclaving for 3 h at 125°C (Van Schreven et al., 1954), and gamma-irradiation at 5×10^6 rad (Roughley and Vincent, 1967; Somasegaran and Halliday, 1982). In order to avoid the production of inhibitory substances during peat sterilization, the temperature should not be more than 100°C. Roughley and Vincent (1967) recommended a temperature of 80 to 100°C.

The sterilized peat is then inoculated with a certain volume of Rhizobium broth culture so that the moisture content of the peat inoculant culture produced is 67 dag kg^{-1} on a dry weight basis (Vincent, 1970). Rhizobium broth culture is usually prepared either in yeast extract mannitol (YEM) solution (Vincent, 1970) or in a defined medium (Bergersen, 1961) and incubated at 30°C for 4 to 5 d on a rotary shaker at 130 cycles min^{-1}. After inoculation, the peat culture is kept for 2 to 3 weeks at 28°C.

For commercial purposes, polythene bags having a thickness of 0.0375 mm are usually used in packaging peat

cultures (Vincent, 1970). Burton (1965) used polythene having a thickness of 0.038 to 0.051 mm. Each bag usually contains 50 to 100 g of moist peat culture.

Viability of Rhizobium in a peat inoculant will be influenced by various factors such as pH, sterilization, storage temperature, moisture content, aeration, NaCl content, and Rhizobium strain. For optimum growth of rhizobia, pH of the peat should be around 6.5 to 7.0. Roughley (1968) found a rapid loss of rhizobial viability in a non-sterile peat. Low storage temperatures are favorable for the survival of rhizobia (Iswaran et al., 1969). Low storage temperatures reduce rhizobial cells desiccation (Van Schreven, 1970). Storage temperature for peat inoculants recommended by Vincent (1970) was 2 to 5°C. Moisture content of 35 to 40 dag kg^{-1} (wet basis) appears to be satisfactory for peat inoculants (Burton, 1967). Free access of air into a carrier material is necessary for good growth of rhizobia (Hedlin and Newton, 1948; Iswaran, 1971). Conversely, Spencer and Newton (1953) claimed satisfactory survival of rhizobia in tightly sealed cans and jars. Sodium chloride content should not be more than 0.2 dag kg^{-1} for good growth of Rhizobium (Steinborn and Roughley, 1974). The inhibitory effect of NaCl on rhizobia has also been reported by Pillai and Sen (1966). Rhizobium in peat inoculated with more than one strain can show antagonistic and competitive effects (Marshall, 1956).

Other inoculant types include solid inoculant, soil-implant inoculant (Fraser, 1966), and granular inoculant (Bezdicek et al., 1978; Muldoon et al., 1980). These inoculant types are made by coating solid or granulated materials with a slurry of peat inoculant. Gum arabic (GA) or methyl cellulose are usually used as stickers. Solid-base inoculants are superior to other inoculant types (Burton and Corley, 1965; Shipton and Parker, 1967; Date, 1968).

As discussed previously, the second factor influencing the success of legume inoculation is the inoculation method. According to Brockwell (1982) there are two inoculation methods: (1) seed inoculation in which inoculant is applied directly to seeds before sowing and (2) seed bed inoculation in which inoculant is applied directly into the seed bed before or after the seeds have been planted. Seed inoculation can be done in three ways: dusting, slurry inoculation, and seed pelleting. Dusting is done by mixing inoculant with seeds in a seed box before sowing. In slurry inoculation, the inoculant is applied to seeds as a water suspension. Sticker may also be mixed in the suspension in this method. In seed pelleting, seeds are mixed with the mixture of sticker and peat inoculant and then coated with pelleting material such as lime ($CaCO_3$).

Among the inoculation methods discussed above, the seed pelleting technique appears to be the preferred method of inoculation (Vincent, 1970; Brockwell, 1982).

Factors, other than inoculant type and inoculation method, that influence the legume inoculation success include Rhizobium strain; legume type; soil acidity; soil nutrients; soil water, O_2 and CO_2; soil temperature; soil organisms; soil toxins and pesticides.

Competition between Rhizobium strains has been reported by Gibson et al. (1976), Marques et al. (1974), Labandera and Vincent (1975), and Winarno and Lie (1979), and others. Labandera and Vincent (1975) demonstrated how TAI strain of Rhizobium out-competed a Uruguayan strain from subterranean clover (Trifolium subterraneum L.).

The magnitude of N_2 fixed by Rhizobium depends on the legume type (Dart, 1974; Alexander, 1977). Alexander (1977) listed the approximate amounts of N that can be fixed in various legumes, as follows (kg N ha^{-1} y^{-1}): alfalfa (Medicago sativa L.), 125 to 335; red clover (Trifolium pratense L.) , 85 to 190; pea (Pisum sativum L.), 80 to 150; soybean (Glycine max (L.) Merr.), 65 to 115; cowpea (Vigna unguiculata (L.) Walp.), 65 to 130 and vetch (Vicia acutifolia L.), 90 to 155).

Soil acidity affects the growth of both Rhizobium and legumes. As soil acidity increases the growth of legumes and the rate of N_2-fixation by Rhizobium decreases. According to Smith (1956), the optimum soil pH for N_2-fixation on lotus ranged from 6.3 to 8.3, according to strain. Munns (1965) reported that an optimum pH for N_2-fixation by alfalfa was 5.5 to 6.0. Andrew (1976) reported that pH for

maximum nodulation on alfalfa, M. truncatula Gaertn., M. scutellata (L.) Mill. and Glycine wightii (Grah. ex Wight & Arn.) Verdc. was 6.0. At pH 4.0 to 5.0, nodulation of Desmodium uncinatum (Jacq.) DC. and Trifolium repens was markedly reduced. Evans et al. (1980) concluded that nodulation and N_2-fixation by Rhizobium leguminosarum and Pisum sativum were optimum at pH 4.8 to 6.6. Effect of soil acidity and growth of host plants and Rhizobium is interrelated with the availability of P and Ca and toxicity of Al and Mn.

Soil nutrients that can influence nodulation and N_2-fixation include N, P, Ca, S, Al, Mn, Mo and Co. Thornton (1956) found the reduction in yield of Roanoke soybean to be due to the reduction in N_2-fixation caused by applying 38 kg NH_4NO_3 ha^{-1}. Bezdicek et al. (1974) reported a reduction in N_2-fixation due to NH_4NO_3 and urea. Ratner et al. (1979) found no significant increase in N_2-fixation using 80 kg N ha^{-1} on inoculated peanut (Arachis hypogaea L.). High levels of soil N usually reduce N_2-fixation; nevertheless, a small amount of N is still needed for nodule development (Lie, 1974). Simpson and Gibson (1970) found that N_2-fixation increased at 30 mg NO_3^- -N kg^{-1}. Phosphorus also has a positive effect on nodulation (Hagedorn, 1979). Keyser and Munns (1979) found that at very low P concentrations, a population of Rhizobium was reduced and some strains could not grow. The increases in N_2-fixation due to S and Mo have been reported by Hagedorn (1979). Reisenauer

(1960) reported that the use of 0.1 μM Mo increased N_2-fixation by alfalfa from 34 mg to 237 mg N per pot.

Nitrogen fixation is influenced by soil water, O_2 and CO_2. Farrington et al. (1977) reported that waterlogging greatly reduced N_2-fixation on lupine (Lupinus diffusus Nutt.). Lie (1974) stated that either too high or too low soil moisture content is detrimental to water uptake by plants and for N_2-fixation. Detrimental effect of water is due to the reduction in O_2 concentrations required around nodules for root respiration (Mulder and Van Veen, 1960).

Soil temperature has a significant influence on nodulation (Vincent, 1958). Weber and Miller (1972) found an increase in nodule number from 92 to 445 per plant on Lee soybean when temperature was increased from 10 to 30°C. Day et al. (1978) reported that the best growth and nodulation of cowpea was at 30 and 36°C. Nodulation was not found at temperatures above 40°C. According to Lie (1974), optimum temperature for clover for N_2-fixation should be around 20°C.

In soils, Rhizobium may be attacked by other soil organisms, such as Bdellovibrio (Keya and ALexander, 1975), bacteriophages (Kowalski et al., 1974), and protozoa (Sardeshpande et al., 1977). Nair (1978) reported that chloropidan larvae can heavily damage nodules of cowpea and crotalaria. Guy et al. (1980) reported that nodule numbers of white clover were markedly reduced due to infection by white clover mosaic virus. Reduction in rhizobial numbers

in all cases discussed above could reduce nodule formation and function.

Holland and Parker (1966) found inhibition of nodulation of subterranean clover by fungal antibiotics. Chatel and Parker (1972) found the inhibition effect of soil-water extract on nodulation of white clover due to the presence of soil toxins. Kandasamy and Prasad (1971) reported that phenolics exuded by roots are also able to decrease rhizobia in soils.

Pesticides may adversely affect nodulation and N_2-fixation. Lie (1974) found an inhibition effect of organophosphorus and carbamate on N_2-fixation at concentrations of 50 and 500 mg kg^{-1} of soil, respectively. At a concentration of 5 mg kg^{-1} of soil, the two pesticides did not inhibit N_2-fixation. Sekar and Balasubramaniam (1979) also reported inhibition of N_2-fixation by carbamate pesticides at concentrations of 5 and 10 mg kg^{-1}, but not at 2 mg kg^{-1}.

The Use of Stickers in Legume Inoculation

Stickers or adhesives are used in legume inoculation to stick *Rhizobium* cells or inoculant on seeds or granulated materials as described previously. Materials commonly used for stickers are gums or natural plant hydrocolloids which may be classified as anionic polysaccharides or salts of polysaccharides (Tyler et al., 1976). According to these authors, the commercially useful adhesives include shrub or

tree exudates such as acacia (Acacia senegal (L.) Willd.), karaya (Sterculia urens Roxb.) and tragacanth (Astragalus gummifer Labill.); marine gums such as agar, algin and carageenan; seed gums such as guar, locust bean and psyllum; plant extracts such as pectins; starch and cellulose derivatives such as hydroxy ethyl starch, carboxy methyl cellulose, ethyl cellulose, hydroxy propyl methyl cellulose, methyl cellulose and oxidized cellulose; and microbial gums such as dextrans and xanthan. Household sugar (sucrose) has also been used as a sticker (Brockwell, 1982). The gum tapped from acacia is also called gum arabic (GA) since this gum was extensively used by the early Arabian physicians (Tyler et al., 1976).

Stickers have been used in slurry inoculation and in soil-implant inoculant formulation as previously described. Stickers such as GA are also used in seed pelleting.

In making pelleted seeds (Roughley et al., 1966), GA is first dissolved, then cooled and chilled overnight. Inoculum is then added to the gum solution and mixed. The gum-inoculum mixture is then added to the seeds and mixed until each seed is evenly coated. Lime is added all at once and mixed rapidly for 1 to 1.5 minutes. The excess lime is removed by sieving if necessary. According to Vincent (1970), each seed should be evenly coated and no seed surface should be visible after pelleting. After pelleting seed, weight can increase as much as 50 to 70% (Brockwell, 1962).

The concentration of the sticker solution depends on the kind of sticker used. Brockwell (1962) used GA and methyl ethyl cellulose solution at a concentration of 45 and 5 dag L^{-1}, respectively. Roughley et al. (1966) recommended 30 dag L^{-1} for GA, while Waggoner et al. (1979) used 42 dag L^{-1} concentration. Volume of GA solution kg^{-1} of legume seed varies depending upon seed size. For each kilogram of seed of white clover, subterranean clover, vetch, and soybean, 40, 20, 10, and 5 ml of GA solution were needed, respectively (Roughley et al., 1966). The weight of peat inoculant that should be mixed with seeds depends upon the desired cell number per seed and the Rhizobium population in the culture itself. According to Burton (1967), 4 g of peat inoculant with a Rhizobium population of 1×10^8 cells g^{-1} is needed to inoculate 1 kg of white clover seeds to give approximately 200 cells per seed (1 kg was assumed to contain 2×10^6 seeds). According to this value, if the Rhizobium population was only 1×10^5 cells g^{-1}, the amount of peat inoculant needed would be 4 kg kg^{-1} of seeds. On the other hand, if the population was 1×10^9 cells g^{-1}, the peat inoculant needed would only be 0.4 g $kg^{-1}$of seeds. Vincent (1974) recommended the uses of 10, 5, and 2.5 g of peat inoculant kg^{-1} seeds of white clover, subterranean clover and vetch, respectively, to provide 300 cells per seed. Waggoner et al. (1979) used 10 g peat inoculant kg^{-1} of white clover to get 600 cells per seed.

Cell numbers per seed recommended in legume inoculation vary from one researcher to another and also depend on seed size. In Australia, at least 100 rhizobia per seed are recommended when conditions for planting and seed germination are optimal (Dale, 1970; Dale, 1976; Roughley, 1976). Burton (1967) recommended 200 cells per seed for white clover. An experiment under field conditions by Waggoner et al. (1979) with white clover showed no significant increase in nodule numbers and plant dry weight from 600 to 3,000 cells per seed. On subterranean clover under field conditions, Brockwell (1962) used 3700 to 5100 cells per seed. According to Vincent (1970), viable cells of Rhizobium at sowing time should be in the range of 1,000 to 10,000 cells per seed. Weaver and Frederick (1972) recommended 2,000 to 100,000 rhizobial cells per soy bean seed for maximum total N_2-fixation. Ireland and Vincent (1968) recommended cell numbers of 100,000 to 1,000,000 per seed, to overcome severe competition by rhizobia already in the soils. The cell number per seed of legumes that have been recommended by researchers range from 100 to 1,000,000.

The weight of fine lime kg^{-1} seeds of white clover, subterranean clover and vetch is 490, 240, and 12 g, respectively (Vincent, 1970). Lime is not the only material used in seed pelleting. Hasting and Drake (1960) listed alternative pelleting materials such as basic slag, superphosphate, clay, and several soil types. Other pelleting materials tested by Brockwell (1962) included dolomite, wood charcoal,

charcoal, vitamins + dolomite, yeast extract + dolomite, milk powder + dolomite, and dried blood + dolomite. These pelleting materials showed effects similar to lime on the percentage of plants forming nodules in the crown of subterranean clover, but lime yielded plant dry weights nearly 2 times that of other pelleting materials. In the presence of only GA, the percentage of plants forming nodules was still significantly higher than uninoculated plants, but there was no difference in the plant dry weight. Seed pelleting materials should pass a 300-mesh sieve (Vincent, 1970).

Few researchers have tested the superiority of using stickers as compared to normal inoculation. Hasting and Drake (1960) used white clover seeds grown on agar slants. As sticker, they used 5 dag methyl cellulose L^{-1}. Methyl cellulose increased number of plants forming nodules by about 21% over normal inoculation; there was a further increase of about 25% in the presence of pelleting material. The percentage of plants forming nodules from pelleted seeds was unchanged after 92 d of storage. Nodulation was not found with normal inoculation or using only sticker after a 92 d storage period. Dry weight of plants from pelleted seeds increased nearly 19 times that of normally inoculated plants for a seed storage period of 42 d. When only sticker was used (without pelleting material), plant dry weight was not increased.

With subterranean clover under field conditions, Brockwell (1962) found no significant difference between pelleted seeds and using only GA on the percentage of plants forming nodules. Both treatments increased a percentage of plants forming nodules by nearly 2.5 times over that of normal inoculation. Plant dry weight showed no significant increase from using only GA but seeds pelleted with lime yielded plant dry weights nearly 2 times that of normal inoculation.

A field experiment with white clover (Waggoner et al., 1979) showed that at 153 d after planting, nodule numbers of tap root for GA with 600 cells per seed (GA-600) were about 2 times that of water using the same cell number per seed (Water-600). Nodule numbers of Water-600 were not significantly different from the uninoculated control. Plant dry weight of GA-600 was about 2 times that of Water-600 but plant dry weight of Water-600 was only about 25% of the control. At 114 d after planting, the plant dry weights of Water-600 were only 60% of the control. The increase in cell numbers per seed from 600 to 3,000 using GA did not significantly increase either nodule numbers or plant dry weight.

Evers (1980) also reported two experiments on the effect of GA on white clover on fine sandy loam and clay soils under field conditions. Fine sandy loam soil gave nodule number for GA which was about 1.5 times that for water. Dry weight of the plants showed no difference

between GA and water. The experiment on clay soil did not show any superiority of GA over water either in nodule numbers or plant dry weight.

How Stickers May Enhance Nodulation

Subba-Rao et al. (1971) studied the effect of GA on nodulation of cluster clover (*Trifolium glomeratum* L.) using agar slopes under bacteriologically controlled conditions. Results of the experiment (Table 1) showed a tendency for GA to enhance number of infected root hairs. Number of infected root hairs at 0.1 g GA L^{-1} was about 3.4 times that of the control but at 2.0 g GA L^{-1} the number of infected root hairs was only about half that for 0.1 g GA L^{-1}. The increase in GA concentrations from 0 to 2 g L^{-1} did not significantly correlate with the increase in nodule numbers and plant dry weight at 60 d after planting.

Summary

The success of legume inoculation depends upon factors which include inoculation method, inoculant type, *Rhizobium* strain, legume type, and soil and environmental conditions. Seed inoculation using stickers and coating with lime as pelleting material appears to be the best inoculation method for legumes. Stickers such as GA and methyl cellulose both enhanced nodulation but few experiments have been done to test the superiority of using stickers over normal inoculation. The reasons why stickers enhance nodulation are

Table 1

Effect of gum arabic (GA) concentrations on number of infected root hairs, nodule number, and plant dry weight of cluster clover*

GA concen-trations	Number of infected root hairs/ 5 roots	Number of nodule premordia/ 5 roots	GA concen-trations	Nodule number/ 20 plants at day 20	Plant dry weight/ 20 plants at day 20
$g\ L^{-1}$			$g\ L^{-1}$		
0	13.2	3.2	0	11.9	7.69
0.01	22.0	4.2	0.01	10.0	8.80
0.1	45.0	2.2	0.05	10.4	7.00
0.5	19.4	6.4	0.1	9.4	7.40
1.0	24.6	5.8	0.5	17.5	8.48
2.0	21.4	5.0	1.0	13.1	8.55
			2.0	12.4	9.08

*From Subba-Rao et al. (1971).

still not clear because there are insufficient experimental data on the subject. Experiments reported by Subba-Rao et al. (1971) indicated that increasing GA concentrations in Jensen's solution increased number of infected root hairs of cluster clover. Further studies are needed on the use of stickers in legume inoculation and how stickers enhance nodulation.

MATERIALS AND METHODS

Experiments on the Use of Stickers to Enhance Nodulation

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Field Conditions

The objective of this experiment was to compare the effects of GA sticker and water on nodulation of white clover under field conditions. This experiment was done on soil of the Arredondo series (Grossarenic Paleudult), on the campus of the University of Florida, Gainesville. Chemical properties of the soil are given in Table 16 in the Appendix. The indigenous population of *Rhizobium trifolii* as determined by a plant infection method (Vincent, 1970) with white clover was $\leq$ 25 cells g^{-1} of moist soil. The experimental design was a randomized complete block with three treatments and five blocks. The treatments were as follows:

A. Control: 0.78 g seeds (about 1180 seeds) + 0.4 g $CaCO_3$.

B. Water as sticker: 0.78 g seeds + 7.2 mg peat inoculant + 0.26 mL water + 0.4 g $CaCO_3$.

C. GA as sticker: 0.78 g seeds + 7.2 mg peat inoculant + 0.26 mL GA solution (45 dag L^{-1}) + 0.4 g $CaCO_3$.

The peat inoculant used was made in the laboratory from unsterilized peat, pH 6.5, inoculated with Rhizobium trifolii strain 0403. This inoculant was used 2 months after preparation. Rhizobium population at inoculation was 9.9×10^7 cells g^{-1} of moist peat. Cell number per seed was approximately 600. Gum arabic used in these studies was TIC (The Integrity Counts) Pretested Arabic 1 (TIC Gums Incorporated, New York). Treatment C was pelleted seeds according to Vincent (1970). After pelleting, weight of seeds increased by about 50%.

Size of experimental plots was 1 x 1 m. Ditches 30 cm wide and 20 cm deep were made between plots. Each plot was fertilized with 20 kg P ha^{-1} in the form of triple superphosphate. Immediately after pelleting, seeds of white clover were planted in four rows on 20 cm centers, at a depth of 1.5 cm and watered. Plants were then hand-weeded periodically and watered as needed.

At 12 weeks after planting, 30 to 40 plants were sampled from each plot. Because of problems of plant growth on one block, plant sampling was done only from four blocks. Roots were cleaned and nodules were counted. Plant tops and roots were dried separately in an oven at 70°C for 24 h. At 16 weeks after planting, the remainder of the plants from each plot were harvested, cleaned, dried, and weighed. Nitrogen concentration of the tops at the 12 weeks of age plants was analyzed by the method of Nelson and Sommers

(1973). Statistical analyses were based on data from four blocks.

Effect of Gum Arabic (GA) Sticker on Nodulation of Centrosema pubescens Under Field Conditions

This experiment was located in the same vicinity as the previous experiment. The experimental design was a randomized complete block in five replicates. Treatments were arranged in a factorial structure of 2 x 2, as follows:

A. 2.4 g seeds (about 100 seeds) + 4.7 mg uninoculated peat + 0.3 mL water + 0.5 g $CaCO_3$.

B. 2.4 g seeds + 4.7 mg uninoculated peat + 0.3 mL GA solution (45 dag L^{-1}) + 0.5 g $CaCO_3$.

C. 2.4 g seeds + 4.7 mg peat inoculant to 0.3 mL water + 0.5 g $CaCO_3$.

D. 2.4 g seeds + 4.7 mg peat inoculant + 0.3 mL GA solution (45 dag L^{-1}) + 0.5 g $CaCO_3$.

In this experiment gamma-irradiated peat (Nitragin Company, Milwaukee, Wisconsin) was used after mixing with sterile $CaCO_3$ to give a pH of 6.5. The amount of $CaCO_3$ used was 5 dag kg^{-1} of the weight of the mixture of the peat and $CaCO_3$. The peat was inoculated with Rhizobium sp. isolated from EL type inoculant (Nitragin Company, Milwaukee, Wisconsin). The population of Rhizobium was 1.27×10^8 cells g^{-1} of moist peat. Cell number per seed was 6,000.

Plot preparation was the same as for the previous experiment. Each plot was fertilized with 100 kg P, 50 kg K

and 30 kg Mg ha^{-1} as triple super phosphate, K_2SO_4, and dolomite, respectively. The seeds were planted in four rows per plot at a depth of 1.5 cm. Daily temperature at a soil depth of 2 cm at the first week after planting was 30 to 34°C. Hand-weeding was done periodically. At 10 weeks after planting, nodule number, nodule dry weight, top dry weight and root dry weight per plant were determined.

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Laboratory Conditions

This experiment was done in the Soil Microbiology Laboratory, Soil Science Department, University of Florida. Treatments were arranged in a factorial structure of 2 x 2 with six replicates, using a randomized complete block design. Treatments were as follows:

A. 0.78 g seeds + 1.8 mg uninoculated peat + 0.26 mL water + 0.4 g $CaCO_3$.

B. 0.78 g seeds + 1.8 mg uninoculated peat + 0.26 mL GA solution (45 dag L^{-1}) + 0.4 g $CaCO_3$.

C. 0.78 g seeds + 1.8 mg peat inoculant + 0.26 mL water + 0.4 g $CaCO_3$.

D. 0.78 g seeds + 1.8 mg peat inoculant + 0.26 mL GA solution (45 dag L^{-1}) + 0.4 g $CaCO_3$.

Rhizobium population of the peat inoculant was 2 x 10^9 cells g^{-1} of moist peat. Cell number per seed was 3,000.

Twenty-four 100-ml beakers were used. Each was filled with 100 g of Arredondo sandy soil. The soil was sampled

from a field behind the previous experiments and placed in a plastic bucket in the field. To lower the level of soil N, the soil was kept in a waterlogged condition for 3 weeks; water above the soil in the bucket was changed several times then dried in the field. In the laboratory, the soil was air-dried and screened through a 2-mm sieve. Chemical properties of the soil are given in Table 16 in the Appendix. After the soil had been placed in the beakers, it was fertilized with 200 mg P, 125 mg K and 125 mg Mg kg^{-1} of soil as $Ca(H_2PO_4)_2$, KCl, and $MgSO_4.7H_2O$, respectively. After watering to 25 dag kg^{-1} of soil, the soil was steam-sterilized for 1 h on 2 successive d.

The seeds were planted at the rate of 14 per beaker at a depth of 0.7 cm. The beakers were placed under 12 h d^{-1} illumination by six warm-white-40 watt flourescent tubes at a distance of 45 cm above the soil. The room temperature fluctuated from 15 to 27°C. The beakers were watered to constant weight daily with sterile water. At 2 weeks after planting, the plants were thinned to five per beaker. At 6 weeks after planting, the plants were harvested. Nodules were counted, and plant tops and roots were separated and oven-dried.

Effect of Gum Arabic (GA) Sticker on Nodulation of Calopogonium caeruleum Under Greenhouse Conditions

Design of experiment, treatments, and the soil used were the same as for the white clover experiment conducted

under laboratory conditions as described previously. Treatments in this experiment were as follows:

A. 1.2 g seeds (about 39 seeds) + 0.9 mg uninoculated peat + 0.4 mL water + 0.3 g $CaCO_3$.

B. 1.2 g seeds + 0.9 mg uninoculated peat + 0.4 mL GA solution (45 dag L^{-1}) + 0.3 g $CaCO_3$.

C. 1.2 g seeds + 0.9 mg peat inoculant + 0.4 mL water + 0.3 g $CaCO_3$.

D. 1.2 g seeds + 0.9 mg peat inoculant + 0.4 mL GA solution (45 dag L^{-1}) + 0.3 g $CaCO_3$.

Population of Rhizobium sp. was 1.27×10^8 cells g^{-1} of moist peat.

Twenty-four clay pots (10.5 cm diameter) were each filled with 350 g of sandy soil. The soil was fertilized with P, K, and Mg as previously described for the white clover experiment under laboratory conditions. After watering to 25 dag kg^{-1} of soil, the pots were autoclaved at 121°C and 11×10^4 Pa for 15 minutes. Seeds of Calopogonium caeruleum were planted at the rate of four per pot at a depth of 1 cm. The daily temperature of the greenhouse was kept at less than 30°C. Pots were watered to constant weight with sterile water. Plants were thinned to one per pot after 2 weeks. At 8 weeks after planting, nodule number, nodule dry weight, top dry weight and root dry weight were recorded. Nitrogen concentration of plant tops was determined according to Nelson and Sommers (1973).

Effect of Different Stickers on Nodulation of White Clover Under Laboratory Conditions

The objective of this experiment was to compare the effect of different stickers on nodulation of white clover under laboratory conditions.

Experimental design was a randomized complete block with nine treatments in three replicates. The treatments were

A. Water

B. TIC Pretested Arabic 1 (45 dag L^{-1})

C. TIC Pretested Arabic 1 (2.5 dag L^{-1})

D. Arabic TIC Spray Dry USP Powder (2.5 dag L^{-1})

E. TIC Pretested Karaya "B" (2.5 dag L^{-1})

F. TIC Pretested Tamarind (2.5 dag L^{-1})

G. TIC Pretested Ghatti (2.5 dag L^{-1})

H. Gum Arabic TIC Pretested (2.5 dag L^{-1})

I. Tapioca Starch (2.5 dag L^{-1})

Plant containers were 100-ml glass beaker, each filled with 100 g of Arredondo sandy soil. The soil used, rates, and forms of fertilizers and watering method were the same as for the previous experiment on white clover under laboratory conditions. Before seeds were planted, the soil was autoclaved for 15 minutes. Volume of each sticker solution was 0.26 mL. This was mixed with 0.78 g seeds, 32 mg peat culture, and 0.4 g $CaCO_3$. Cell number per seed was 600. Rhizobium population of the peat inoculant was 2.2×10^7 cells g^{-1} of moist peat. Fourteen seeds were planted per

beaker and plants were thinned to five per beaker after 2 weeks. The experiment was conducted in a growth chamber with an illumination period of 12 h d^{-1} and temperature of 25° to 27°C. Source of light was six warm-white-60 watt flourescent tubes at a distance of 55 cm above the soil. Nodule number, top dry weight, and root dry weight were recorded at 6 weeks after planting. Differences between treatments were tested by Duncan's Multiple Range Test (Duncan, 1955).

Experiments to Explain How Gum Arabic (GA) Sticker Enhances Nodulation

Effect of Gum Arabic (GA) on the Growth of Rhizobium in a Defined Medium

The objective was to study the effect of increasing gum arabic concentrations on *Rhizobium* cell numbers in a defined medium.

Experimental design was a randomized complete block with five treatments and three replicates. Unlike the previous experiments, blocking in this experiment was a time-difference to run each set of treatments. Period of each incubation was 1 week. The treatments were

A. Defined medium pH 6.9 (10 g mannitol L^{-1})

B. Defined medium minus mannitol (0 g GA L^{-1})

C. Defined medium plus 5 g GA L^{-1}

D. Defined medium plus 25 g GA L^{-1}

E. Defined medium plus 50 g GA L^{-1}

Five 250 mL Erlenmeyer side-arm flasks were used for each set of treatments. Each flask was filled with 100 ml of a defined medium (Bergersen, 1961). Treatments C, D, and E were adjusted to pH 6.9 with a sterile solution of 0.1 M NaOH. Gum arabic (TIC Pretested Arabic 1) was added to the flasks before adding the defined medium. Each flask was inoculated with 1 mL of 7 d-broth culture of Rhizobium trifolii strain 0403 (3×10^8 cells mL^{-1}). The flasks were incubated for 7 d at 30°C on a rotary shaker at 130 cycles $min.^{-1}$. At day 7, cell numbers were counted using pour plates (Clark, 1965) and the Petroff-Hausser method (Vincent, 1970).

Duncan's Multiple Range Test was used to compare differences between treatments. Data for treatments B to E were also analyzed separately to study the relationship between increasing GA concentrations and Rhizobium cell numbers.

Effect of Gum Arabic (GA) on Nodulation of White Clover Grown in Fahraeus' Medium

The objective was to study the effect of GA on early nodulation of white clover in a solution culture (Fåhraeus' medium).

Experimental design was a randomized complete block with two treatments and 10 replicates. The treatments were

A. Control (0 g GA L^{-1})

B. 5 g GA L^{-1}

Twenty large glass tubes (3.5 cm diameter and 11 cm high) were used. Each was filled with 35 mL of Fåhraeus' medium (Fåhraeus, 1957). The medium was adjusted to pH 6.5 with sterile 0.1 M NaOH after autoclaving the medium. One aseptically germinated white clover seedling was transferred to each tube using a technique described by Fåhraeus (1957). The seedling was placed between a glass slide and a cover slide. Size of glass slide was 7.5 x 2.5 cm and cover slide was 5 x 2.5 cm. The tubes were inoculated with 1 mL of 5 d broth culture of Rhizobium trifolii strain 0403 (35,000 cells mL^{-1}) to give 1000 cells mL^{-1} of the medium. The tubes were placed under illumination of six warm-white-40 watt fluorescent tubes for a period of 12 h d^{-1}. Plants were observed daily until nodules had developed and nodule counts were made every 3 d thereafter until 15 d after inoculation.

Effect of Gum Arabic (GA) Concentrations in Soil on Nodulation of White Clover Under Laboratory Conditions

The objective of this experiment was to study the effect of increasing GA concentrations on nodulation of white clover in soil under laboratory conditions. The soil used was the same as for the previously described experiments on white clover under laboratory conditions.

Experimental design was a randomized complete block with six treatments and four replicates. The treatments were

A. 1.4 dag GA kg^{-1}, uninoculated.

B. 0 dag GA kg^{-1}, inoculated with Rhizobium.

C. 0.7 dag GA kg^{-1}, inoculated with Rhizobium.

D. 1.4 dag GA kg^{-1}, inoculated with Rhizobium.

E. 2.1 dag GA kg^{-1}, inoculated with Rhizobium.

F. 2.8 dag GA kg^{-1}, inoculated with Rhizobium.

Plants were grown in 50-mL beakers filled with 50 g soil previously autoclaved for 30 minutes at 121°C and 11 x 10^4 Pa (16 lb in^{-2}). Phosphorus, K, and Mg were applied to the soil as previously described. Gum arabic was dissolved in 4 mL distilled water and autoclaved for 15 minutes before it was mixed with the soil. Water content of the soil was kept at 25 dag kg^{-1} of soil. Seven surface-sterilized seeds (Vincent, 1970) were planted in each beaker at a depth of 0.7 cm. Inoculation was done with 1 mL of Rhizobium culture (3 x 10^8 cells mL^{-1}) grown in a defined medium for 5 d. At 2 weeks after planting, the plants were thinned to four per beaker. Observation on one replicate at 4 weeks after inoculation did not show any nodules; therefore, each treatment was reinoculated with the same strain of Rhizobium. At 8 weeks after the second inoculation, nodule number and top and root dry weights were recorded.

Because of significant decreases in the parameters under study in this experiment from GA concentrations of 0.7 dag to 2.8 dag kg^{-1}, another experiment was set up using GA concentrations from 0 to 1.00 dag kg^{-1} and 100 mL beakers filled with 100 g of sandy soil. The experimental design

was a randomized complete block with five treatments and four replicates. The treatments were 0, 0.25, 0.50, 0.75 and 1.00 dag GA kg^{-1} of soil. Seven aseptically germinated seedlings were planted in each beaker. At 6 weeks after planting, nodule number and plant top and root dry weight were recorded.

Effect of Rhizobium Cell Number per Seed on Nodulation of White Clover

The objective was to test the effect of increasing cell number of *Rhizobium* on nodulation using water as sticker.

Experimental design was a randomized complete block with six treatments and three replicates. The treatments were

A. GA sticker, 3,000 *Rhizobium* cells per seed (1.8 mg peat/0.78 g seeds).

B. Water, 0 *Rhizobium* cell per seed (0 mg peat/0.78 g seeds).

C. Water, 3,000 *Rhizobium* cells per seed (1.8 mg peat/0.78 g seeds).

D. Water, 6,000 *Rhizobium* cells per seed (3.6 mg peat/0.78 g seeds)

E. Water, 60,000 *Rhizobium* cells per seed (36 mg peat/0.78 g seeds)

F. Water, 180,000 *Rhizobium* cells per seed (108 mg peat/0.78 g seeds).

The expected *Rhizobium* population was 2×10^9 cells g^{-1} of peat inoculant.

Clay pots (10.5 cm diameter) were filled with 350 g Arredondo sandy soil. Rates and forms of fertilizers were the same as in previous laboratory experiments. After watering to 25 dag kg^{-1} of soil, the pots were autoclaved for 15 minutes, cooled, and seeds were planted at the rate of 32 per pot. This experiment was placed in a controlled-environment chamber and watered as in previous laboratory experiments. Nodule number per plant and plant top and root dry weight were recorded at 8 weeks after planting.

Numbers of Rhizobium on the seed surface using either water or GA as sticker were obtained by plate count. Because the actual cell numbers were much lower than the expected values, the counting was repeated using a freshly made peat inoculant having a Rhizobium population of 1.62×10^9 cells g^{-1}.

Effect of Gum Arabic (GA) on Pectinase and Cellulase Production by Rhizobium and of White Clover Seedlings

The first experiment was to study the effect of increasing GA concentrations in a defined medium (Bergersen, 1961) on pectinase and cellulase production by Rhizobium. The treatments were as follows:

A. Defined medium plus 10 g mannitol L^{-1}

B. Defined medium minus mannitol (0 g GA L^{-1})

C. Defined medium plus 5 g GA L^{-1}

D. Defined medium plus 25 g GA L^{-1}

The experimental design was a randomized complete block with eight replicates. These media were inoculated with

Rhizobium and incubated for 7 d. At day 7, 10-mL aliquots of each treatment were centrifuged at 4°C at 5,000 x g for 30 minutes (Martinez-Molina et al., 1979). The clear supernatant of each treatment was used to assay for pectinase and cellulase activities.

The assay for pectinase activity was done according to Fåhraeus and Ljunggren (1959), using 5 mL of pectin solution (2 dag L^{-1}), 2 mL of 0.1 M acetate buffer (pH 5.0), 1 mL of NaCl solution (5 dag L^{-1}) and 3 mL of cell-free culture fluid. Efflux times for t_w, t_o, and t_{24} were observed at 30°C (t_w was efflux time using distilled water, t_o was efflux time at zero time, and t_{24} was efflux time at 24 h). The viscometers were kept in a water bath at 30°C during the assay. Percentage decrease in viscosity (PDV) was calculated as follows:

$$PDV = \frac{t_o - t_{24}}{t_o - t_w} \times 100$$

A 2.0 dag pectin L^{-1} solution was used in the first four replicates. In the second four replicates, 0.2 dag pectin L^{-1} was used because a high coefficient of variability (CV) was found with the first pectin solution concentration.

Cellulase activity was assayed using 0.2 dag carboxyl methyl cellulose (CMC) L^{-1} in phosphate-citric buffer pH 5.0 as substrate (Morales, 1981). Volume of the substrate was

5 mL and volume of the cell-free culture fluid was 1 mL (Scala et al., 1981).

Pectinase and cellulase activities were also assayed by incubation of pectin- and CMC-agar media with sterile paper disks saturated with Rhizobium culture grown in the medium of each treatment. The pectin-agar medium contained (in g L^{-1}): pectin, 2.5.; mannitol, 1.0; yeast extract, 0.1 (Hubbell et al., 1978); K_2HPO_4, 0.5; Mg SO_4 .$7H_2O$, 0.1; NaCl, 0.2; $CaCl_2$.$2H_2O$, 0.2; $FeCl_3$.$6H_2O$, 0.1; agar, 15 (Jayasankar and Graham, 1970). The medium was adjusted to pH 7.0 with 0.1 M NaOH. Volume of the medium per plate was 12 mL. Period of incubation was 3 weeks at 28°C, after which the unhydrolyzed pectin was precipiated with hexadecyltrimethyl ammonium bromide solution (2 dag L^{-1}). The CMC-agar medium was prepared in a similar manner as the pectin-agar medium. Two grams CMC L^{-1} was used instead of 2.5 g pectin L^{-1}. This medium was adjusted to pH 7.0 with M HCl. The plates were developed with Congo red (1 mg L^{-1}), M NaCl and M HCl (Teather and Wood, 1982).

The purpose of the second experiment was to study the effect of GA on pectinase and cellulase activities produced by Rhizobium and white clover seedlings growing in sterile water (Fåhraeus and Ljunggren, 1959). Experimental design was a randomized complete block with eight treatments and three replicates. The treatments were arranged in a factorial structure of 2 x 2 x 2 as follows:

A. Control (Sterile water)

B. GA

C. S (White clover seedlings)

D. S + GA

E. R (Rhizobium)

F. R + GA

G. R + S

H. R + S + GA

Eight Petri dishes were used for each replicate. Treatments C, D, G, and H were planted with 1 g of sterile white clover seed per plate. Five milliliter sterile tap water were placed in Petri dishes for treatments A, C, E, and G and 5 mL of 5 g GA L^{-1} were placed in Petri dishes of treatments B, D, F, and H. The Petri dishes were incubated at 28°C for 48 h. After 48 h, Petri dishes of E, F, G, and H were incubated with 1 mL each of dilute fresh Rhizobium culture grown in a defined medium to give a population of approximately 1300 cells per seed. One milliliter of sterile tap water was also added to Petri dishes of A, B, C, and D. Another 5 mL of 0 g GA L^{-1} was added to Petri dishes A, C, E, and G and 5 mL of 5 g GA L^{-1} was added Petri dishes B, D, F, and H. After inoculation, the Petri dishes were placed in a controlled cabinet using a lighting period of 12 h d^{-1}. After 48 h, each culture was washed two times with 5 mL of sterile tap water and centrifuged for 30 minutes at 5,000 x g at 4°C. The assays for pectinase and cellulase activities were done as described previously using pectin and CMC solutions, respectively.

RESULTS

Experiments on the Use of Stickers to Enhance Nodulation

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Field Conditions

Nodule number, top dry weight, N concentration in tops, and root dry weight at 12 weeks after planting, and plant dry weight at 16 weeks after planting are given in Table 2. Nodule number, top dry weight, and root dry weight for GA sticker at 12 weeks after planting was 32, 28, and 22% higher than that for water, respectively, but these were not significant at $P = 0.05$. The difference in N concentration of the plant tops between treatments was also not significant. At 16 weeks after planting, the increase of plant dry weight due to GA was only 7% more than that for water.

Effect of Gum Arabic (GA) Sticker on Nodulation of Centrosema pubescens Under Field Conditions

Results of this experiment are given in Table 3. The effect of treatments on nodule number per plant, nodule dry weight, top dry weight, and root dry weight were not

Table 2

Effect of gum arabic (GA) sticker on nodule number, plant dry weight, and N concentration of plant tops of white clover under field conditions

Treatments	Nodule number at 12 weeks after planting	Top dry weight at 12 weeks after planting	Root dry weight at 12 weeks after planting	N concentration of the plant top at 12 weeks after planting	Top and root dry weight at 16 weeks after planting
	nodules $plant^{-1}$	mg $plant^{-1}$	mg $plant^{-1}$	dag kg^{-1}	g m^{-2}
A. Control	50	206	62	4.15	154.2
B. Inoculated, water as sticker	59	337	86	4.21	197.9
C. Inoculated, GA as sticker	78	432	105	4.13	212.2
SE	9.9 (NS)	105.4 (NS)	20.4 (NS)	0.09 (NS)	39.5 (NS)

Note: These data were the average of four blocks. Nodule number, top and root dry weights at 12 weeks after planting were the average of 30-40 sampling plants. Data at 16 weeks after planting were the average of the whole plants left on each plot plus plant dry weight at 12 weeks after planting. NS represents not significant and SE represents the standard error.

Table 3

Effect of gum arabic (GA) sticker and inoculation on nodule number, nodule dry weight, top dry weight, and root dry weight of *Centrosema pubescens* grown under field conditions at 10 weeks after planting

Treatments	Nodule number	Nodule dry weight	Top dry weight	Root dry weight
	nodules $plant^{-1}$	mg $plant^{-1}$	g $plant^{-1}$	g $plant^{-1}$
A. Uninoculated, water as sticker	4.09	16.5	8.27	2.15
B. Uninoculated, GA as sticker	1.91	7.5	7.14	1.20
C. Inoculated, water as sticker	3.64	12.1	8.13	2.39
D. Inoculated, GA as sticker	5.52	23.3	6.67	1.83
SE	2.01	9.4	0.74	1.33

Note: These data were the average of five replicates. Differences in the effect of treatments on the parameters under study were not significant.

significant. Nodule number per plant ranged only from 1.9 to 5.5 with nodule dry weight ranged from 7.5 to 23.3 mg per plant. The GA treatment inoculated with Rhizobium produced a trend of more and bigger nodules compared with other treatments.

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Laboratory Conditions

Gum Arabic treatment of inoculated plants (Table 4) resulted in nodule number 1.6 times that of water ($P < 0.05$). Top dry weight for GA treatment of inoculated plants was 15% less than for water, but this was not significant at $P = 0.05$. Top and root dry weights of the uninoculated plants in the GA treatment were 27 and 34%, respectively, less than for water ($P < 0.05$).

Effect of Gum Arabic (GA) Sticker on Nodulation of Calopogonium caeruleum Under Greenhouse Conditions

Interaction effects between sticker and inoculation (Table 5) were highly significant for nodule number, nodule dry weight, top dry weight, root dry weight, and N concentration of plant tops. Nodule number, nodule dry weight, top dry weight per plant, and N concentration of the plant tops for GA were 2.0, 2.4., 2.1, and 1.3 times that for water, respectively ($P < 0.05$ for all four parameters).

Table 4

Effect of gum arabic (GA) sticker and inoculation on nodule number, top dry weight, and root dry weight of white clover grown under laboratory conditions at 6 weeks after planting

Treatments	Nodule number	Top dry weight	Root dry weight
	nodules plant^{-1}	mg plant^{-1}	mg plant^{-1}
A. Uninoculated, water as sticker	0.11	19.5	5.6
B. Uninoculated, GA as sticker	0.98	14.3	3.7
C. Inoculated, water as sticker	2.93	20.5	5.9
D. Inoculated, GA as sticker	4.68	17.5	5.3
LSD (0.05)	1.25	3.9	1.6

Note: These data were the average of four replicates. For each treatment, they were the average of five to seven plants per beaker. Interaction effect between sticker versus inoculation was not significant.

Table 5

Effect of gum arabic (GA) sticker and inoculation on nodule number, nodule dry weight, top dry weight, root dry weight, and N concentration of plant tops of *Calopogonium caeruleum* grown under greenhouse conditions at 8 weeks after planting

Treatments	Nodule number	Nodule dry weight	Top dry weight	Root dry weight	N concentration of plant tops
	nodules plant^{-1}	mg plant^{-1}	mg plant^{-1}	mg plant^{-1}	dag kg^{1}
A. Uninoculated water as sticker	0	0	1042	276	2.17
B. Uninoculated, GA as sticker	0.25	0.8	873	272	2.03
C. Inoculated, water as sticker	76.5	50.5	636	190	3.21
D. Inoculated, Ga as sticker	153.3	122.5	1314	336	4.06
LSD (0.05)	22.3	19.3	354	80	0.53

Note: These data were the average of four replicates. Only one plant of each treatment was kept growing from week 3 to week 8.

Effect of Different Stickers on Nodulation of White Clover Under Laboratory Conditions

The seven stickers used, including tapioca starch, showed a similar effect (Table 6) on nodule number, top and root dry weights per plant at the concentration of 2.5 dag L^{-1} at 6 weeks after planting under laboratory conditions. The data did not show a significant superiority of using stickers over water. There was also no significant difference between GA at concentrations of 45 and 2.5 dag L^{-1} although nodule number for GA at 45 dag L^{-1} was about 26% higher than for 2.5 dag L^{-1}. Gum arabic sticker at 45 dag L^{-1} concentration also gave about 16% higher nodule number per plant than did water.

Experiments to Explain How GA Sticker Enhances Nodulation

Effect of Gum Arabic (GA) on the Growth of Rhizobium in a Defined Medium

Increasing GA concentrations from 0 to 50 g L^{-1} increased cell number significantly from 1.06×10^8 to 13.81×10^8 cells mL^{-1} at 7 d after inoculation (Table 7). The relationship between the increase in GA concentration and cell number (pour plate) is plotted in Fig. 1. Maximum cell number was reached at GA concentration of 46.4 g L^{-1}. This experiment also showed that cell number counted directly by microscopy was about 2 to 3 times those obtained by plate counting, but the first method gave a slightly higher CV value than the second method.

Table 6

Effects of different commercially used stickers on nodule number, top dry weight, and root dry weight of white clover under laboratory conditions at 6 week after planting

Treatments	Nodule number	Top dry weight	Root dry weight
	nodules $plant^{-1}$	mg $plant^{-1}$	mg $plant^{-1}$
A. Water	3.8	23.9	5.6
B. TIC Pretested Arabic 1* (45 dag L^{-1})	4.4	25.6	5.1
C. TIC Pretested Arabic 1 (2.5 dag L^{-1})	3.5	24.2	5.3
D. Arabic TIC Spray Dry USP Powder (2.5 dag L^{-1}	3.5	26.5	5.4
E. TIC Pretested Karaya "B" (2.5 dag L^{-1})	3.6	25.7	5.5
F. TIC Pretested Tamarind (2.5 dag L^{-1})	3.3	26.8	5.5
G. TIC Pretested Ghatti (2.5 dag L^{-1})	3.7	27.6	5.7
H. Gum Arabic TIC Pretested (2.5 dag L^{-1})	3.7	23.8	4.6
I. Tapioca Starch (2.5 dag L^{-1})	3.8	21.0	4.0
SE	0.5 (NS)	3.0 (NS)	0.7 (NS)

*Trade name for GA.

Note: These data were the average of five plants per beaker and the average of three replicates.

Table 7

Effect of gum arabic (GA) concentrations (0 to 50 g L^{-1}) on *Rhizobium* cell number in a defined medium at 7 d after inoculation

Treatments	Plate counting method	Microscopic method using Petroff-Hausser chamber
	cell number mL^{-1} (x 10^{-8})	
A. 10 g mannitol L^{-1}	0.78^{a}	2.66^{a}
B. 0 g GA L^{-1}	1.06^{a}	2.09^{a}
C. 5 g GA L^{-1}	5.44^{b}	17.45^{b}
D. 25 g GA L^{-1}	10.82^{c}	22.09^{b}
E. 50 g GA L^{-1}	13.81^{d}	29.07^{c}
CV (%)	30	38

Note: These data were the average of three replicates. Cell number of each treatment for plate counting method were the average of five plates and for microscopic method were the average of 20 fields. The same letters for each column denote no significant difference between treatments at P = 0.05 using Duncan's Multiple Range Test.

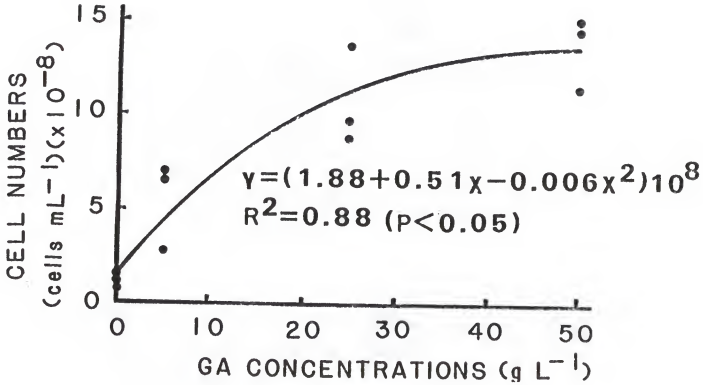

Fig. 1. Relationship between gum arabic (GA) concentrations and *Rhizobium* cell numbers.

Effect of Gum Arabic (GA) on Nodulation of White Clover Grown in Fahraeus' Medium

Results of this experiment are given in Fig. 2. These data showed that nodule number for 5 g GA L^{-1} treated plants was 1.5 times the nodule number for the control ($P < 0.05$) 15 d after inoculation.

Effect of Gum Arabic (GA) Concentrations in Soil on Nodulation of White Clover Under Laboratory Conditions

Two experiments were set up in this study. Results of the first experiment are given in Table 8. The relationships between increasing GA concentrations and nodule number, top dry weight and root dry weight are plotted in Figs. 3, 4 and 5. Results of the second experiment are plotted in Figs. 6, 7, and 8.

Data in Table 8 showed that at the same GA concentration (1.4 dag kg^{-1}), the inoculation increased nodule number, top dry weight and root dry weight. Increasing GA concentrations from 0 to 0.7 dag kg^{-1} increased those three parameters measured but the increases were not significant statistically. From 0.7 to 2.8 dag GA kg^{-1}, the three parameters decreased again and reached their lowest points at 2.8 dag GA kg^{-1}. Based on the equations given in Figs. 3, 4 and 5, the calculated concentrations of GA for its maximum effect on the increase in nodule number, top dry weight, and root dry weight at 12 weeks after planting were 0.6, 1.2 and 1.2 dag kg^{-1}, respectively.

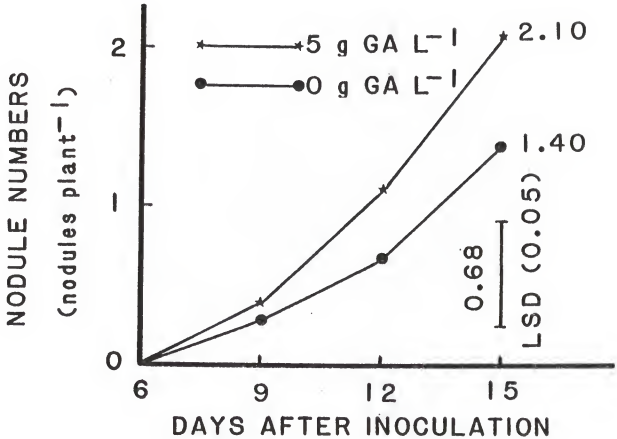

Fig. 2. Effect of gum arabic (GA) on nodulation of white clover grown in Fahraeus' medium.

Table 8

Effect of gum arabic (GA) concentrations (0 to 2.8 dag kg^{-1} of soil) on nodule number, top dry weight, and root dry weight of white clover grown under laboratory conditions at 12 weeks after planting

Treatments	Nodule number	Top dry weight	Root dry weight
	nodules $plant^{-1}$	mg $plant^{-1}$	mg $plant^{-1}$
A. 1.4 dag GA kg^{-1}, uninoculated	3.7^{e}	$41.3^{c,d}$	10.7^{a}
B. 0 dag GA kg^{-1}, inoculated	12^{ab}	48.3^{abcd}	7.9^{bc}
C. 0.7 dag GA kg^{-1}, inoculated	24.7^{a}	60.5^{a}	10.3^{ab}
D. 1.4 dag GA kg^{-1}, inoculated	19^{ab}	56.7^{ab}	9.7^{ab}
E. 2.1 dag GA kg^{-1}, inoculated	16.7^{abcd}	53.8abc	9.3^{ab}
F. 2.8 dag GA kg^{-1}, inoculated	8.7^{cde}	37.9^{d}	5.9^{c}

Note: These data were the average of three replicates. Data for each treatment were the average of four plants per beaker. The same letters in each column denote no significant difference between treatment using Duncan's Multiple Range Test at $P = 0.05$.

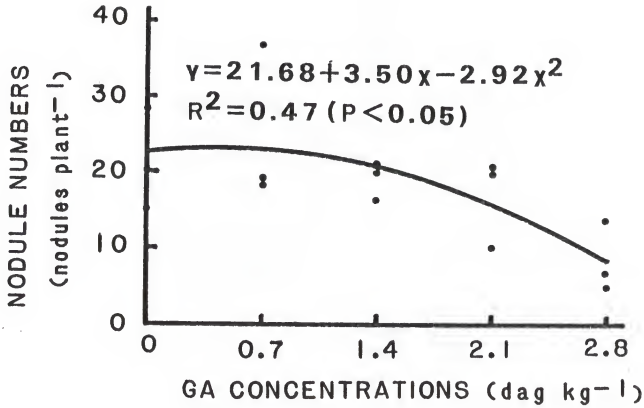

Fig. 3. Relationship between gum arabic (GA) concentrations (0 to 2.8 dag kg^{-1} of soil) and nodule numbers of white clover at 12 weeks after planting.

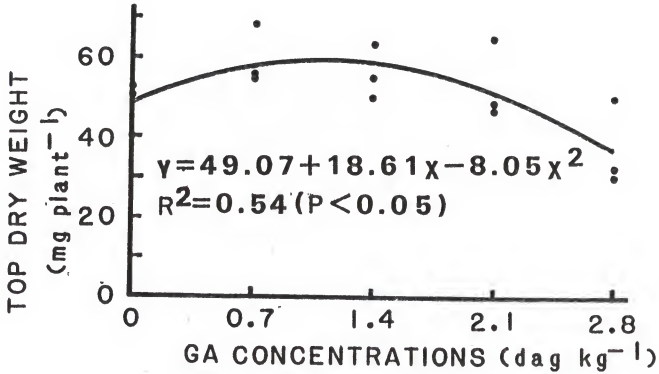

Fig. 4. Relationship between gum arabic (GA) concentrations (0 to 2.8 dag kg^{-1} of soil) and top dry weight of white clover at 12 weeks after planting.

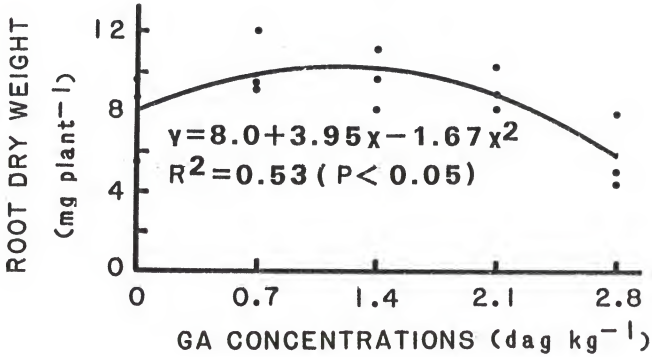

Fig. 5. Relationship between gum arabic (GA) concentrations (0 to 2.8 dag kg^{-1} of soil) and root dry weight of white clover at 12 weeks after planting.

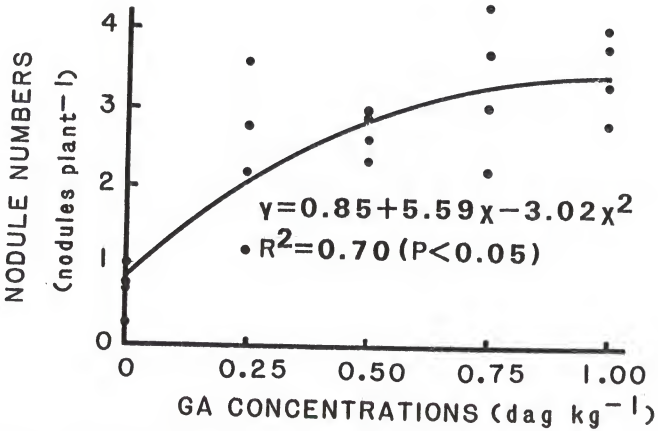

Fig. 6. Relationship between gum arabic (GA) concentrations (0 to 1.00 dag kg^{-1} of soil) and nodule numbers of white clover at 6 weeks after planting.

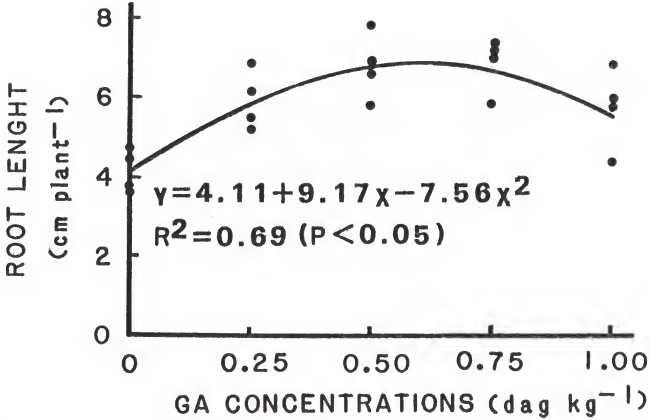

Fig. 7. Relationship between gum arabic (GA) concentrations (0 to 1.00 dag kg^{-1} of soil) and root length of white clover at 6 weeks after planting.

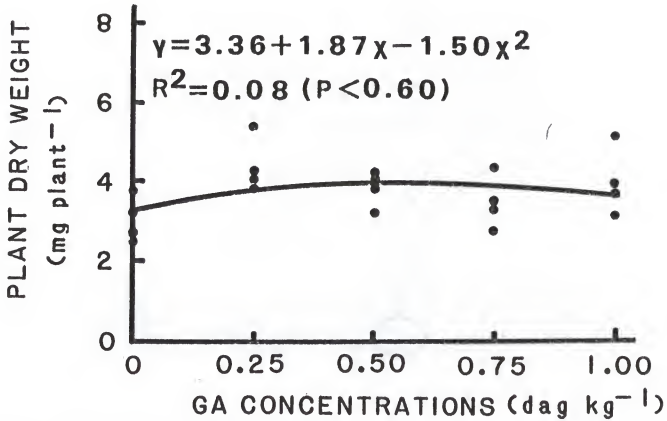

Fig. 8. Relationship between gum arabic (GA) concentrations (0 to 1.00 dag kg^{-1} of soil) and plant dry weight of white clover at 6 weeks after planting.

Figures 6 and 7 show that the calculated GA concentrations for maximum effects on nodule number and the root length at 6 weeks after planting were 0.93 and 0.61 dag kg^{-1}, respectively. This experiment did not show any evidence of increase in plant dry weight due to the increase in GA concentration (Fig. 8).

Effect of Increasing Rhizobium Cell Number per Seed on Nodulation of White Clover

At 3,000 cells per seed, nodule number for GA was 25% higher than for water (Table 9). This was not statistically significant. The increase in cell numbers to about 60 fold, using water as the sticker, did not significantly increase nodule number. Top and root dry weights did not show significant differences between any of the treatments, although no nodules were found for the control (water, 0 cell per seed).

Determinations of actual cell numbers of *Rhizobium* on seeds where the cells were applied with water or GA as sticker are given in Tables 10 and 11. The average GA/W ratio of actual cell number per seed was 2.50 to 2.96 or about 2.5 to 3.0. Determination of actual cell number per seed using a 5-month-old peat inoculant (Table 10) showed that water stuck only about 13% of the expected cell number per seed and GA stuck only 31%. Using a fresh culture, water stuck about 39% while GA stuck nearly 100% of the expected cell number per seed (Table 11).

Table 9

Effect of *Rhizobium* cell number per seed of white clover on nodule number, top dry weight, and root dry weight of plants grown under laboratory conditions at 8 weeks after planting

Treatments	Nodule number	Top dry weight	Root dry weight
	nodules plant^{-1}	mg plant^{-1}	mg plant^{-1}
A. G.A. sticker, 3,000 cells per seed	39.0[b]	52.4	15.4
B. Water, 0 cell per seed	0 [a]	47.2	16.6
C. Water, 3,000 cells per seed	31. [b]	61.5	18.6
D. Water, 6,000 cells per seed	45.3[b]	65.4	19.5
E. Water, 60,000 cells per seed	43.1[b]	57.4	16.9
F. Water, 180,000 cells per seed	43.3[b]	54.7	15.6
SE		6.3	2.5
		(NS)	(NS)

Note: These data were the average of three replicates. Data for each replicate were the average of 20 to 32 plants per pot. The same letters for nodule number denote no significant difference between treatments using Duncan's Multiple Range Test at P = 0.05.

Table 10

Actual *Rhizobium* cell number per seed of white clover found, using water (W) and gum arabic (GA) as stickers (Determination 1)

Peat inoculated*	Cell number			Ratio		
	Expected (E)	In Water (W)	In GA	W/E	GA/E	GA/W
mg	---- cells $seed^{-1}$ ($x10^{-3}$) -----					
1.8	3	0.44	0.50	0.15	0.17	1.14
3.6	6	0.67	1.80	0.11	0.30	2.69
36	60	8.24	20.92	0.14	0.35	2.54
108	180	21.26	77.00	0.12	0.43	2.62
Average				0.13	0.31	2.50

*Mixed with 0.78 g seed.

Note: Actual cell number per seed were the average of 100 seeds. *Rhizobium* population was 2×10^9 cells g^{-1} (based on cell counting).

Table 11

Actual *Rhizobium* cell number per seed of white clover found, using water (W) and gum arabic (GA) as stickers (Determination 2)

Peat inoculated*	Cell number			Ratio		
	Expected (E)	In Water (W)	In GA	W/E	GA/E	GA/W
mg	---- cells seed^{-1} (x10^{-3}) -----					
2.2	3	1.03	3.28	0.34	1.09	3.18
4.4	6	3.43	7.26	0.57	1.21	2.12
44	60	15.96	61.15	0.27	1.02	3.83
132	180	66.67	180.60	0.37	1.00	2.71
Average				0.39	1.08	2.96

*Mixed with 0.78 g seed.

Note: Actual cell number per seed were the average of 50 seeds. *Rhizobium* population was 1.62 x 10^9 cells g^{-1} (based on cell counting).

Effect of Gum Arabic (GA) on Pectinase and Cellulase Production by Rhizobium and White Clover Seedlings

Results of experiments are given in Tables 12, 13, 14, and 15. Pectinase and cellulase activities could not be easily detected in pectin- and CMC-agar media using the procedures outlined in the previous chapter.

The increase in GA concentration in a defined medium did not significantly increase the PDV in pectin solutions (2.0 and 0.2 dag L^{-1}). However, there was a tendency for PDV to increase with increasing concentrations of GA from 0 to 5 g L^{-1} (Table 12). Percentages of decrease in viscosity for the uninoculated treatments were about the same as for the inoculated ones. The increase of GA concentrations from 0 to 5 g L^{-1} increased the PDV in CMC solution (0.2 dag L^{-1}), but again the PDV values of the inoculated treatments were not much different from the uninoculated ones (Table 13).

Table 14a shows that GA alone contributed substantially to the PDV. Gum arabic seemed to induce the production of pectinase by white clover seedlings. Rhizobium did not increase the PDV; white clover seedlings and GA increased the PDV significantly. Based on Table 14b, it was estimated that Rhizobium, white clover seedlings, and GA contributed 8, 33, and 59%, respectively, of the total amount of pectinase production in the culture solution.

Table 12

Effect of gum arabic (GA) in a defined medium on percentage decrease in viscosity (PDV) in 2.0 dag and 0.2 dag pectin L^{-1} in 24 h

Treatments	PDV in 2.0 dag pectin L^{-1}	PDV in 0.2 dag pectin L^{-1} (a)	(b)
A. 10 g mannitol L^{-1}	7.8	9.9	2.9
B. 0 g GA L^{-1}	9.7	6.3	11.1
C. 5 g GA L^{-1}	18.5	7.6	4.3
D. 25 g GA L^{-1}	10.6	5.6	4.2
LSD (0.05)	(NS)	(NS)	3.9
SE	5.1	2.5	
CV(%)	61	48	44

(a) Inoculated with Rhizobium.
(b) Uninoculated.

Note: These data were the average of four replicates. The PDV in pectin solution was assumed due to pectinase activity.

Table 13

Effect of gum arabic (GA) in a defined medium on percentage decrease in viscosity (PDV) in 0.2 dag carboxy methyl cellulose (CMC) L^{-1} in 24 h

Treatments	PDV: (a)	(b)
A. 10 g mannitol L^{-1}	4.8	4.1
B. 0 g GA L^{-1}	7.2	5.0
C. 5 g GA L^{-1}	9.7	10.4
D. 25 g GA L^{-1}	7.1	6.7
LSD (0.05)	2.5	4.3
CV (%)	33	41

(a) Inoculated with Rhizobium.
(b) Uninoculated.

Note: The PDV for (a) and (b) were the average of eight and four replicates, respectively. The PDV in CMC solution was assumed due to cellulase activity.

Table 14

Effects of Rhizobium (R), white clover seedlings (S), and gum arabic (GA) on percentage decrease in viscosity (PDV) in 0.2 dag pectin L^{-1} in 24 h

14a Effects of Combination Treatments of R, S and GA on PDV:

Treatments	Replication 1	2	3	Average
A. Control	9.1	0	6.7	5.3^{ab}
B. GA	25.0	39.3	17.6	27.3^{bc}
C. S	0	0	9.8	3.3^{a}
D. GA + S	87.0	84.0	66.0	79.0^{d}
E. R	3.1	6.7	21.4	10.4^{abc}
F. R + GA	33.3	48.0	15.2	32.2^{c}
G. R + S	0	5.4	34.1	13.2^{abc}
H. R + S + GA	85.2	80.0	94.1	86.4^{d}
SE				9.9

14b Average effects of R, S and GA on PDV*:

Treatments	PDV	Treatments	PDV	Treatments	PDV.
Without R	28.7	Without S	18.8	Without GA	8.0
With R	35.5	With S	45.5	With GA	56.2
LSD (0.05)	NS		10.4		10.4

*Main effects of R, S, and GA = 6.8 (NS), 26.7, and 48.2, respectively.

Note: The same letters in 14a denote no significant difference between treatments using Duncan's Multiple Range Test at P = 0.05.

Table 15

Effects of Rhizobium (R), white clover seedlings (S), and gum arabic (GA) on percentage decrease in viscosity (PDV) in 0.2 dag carboxy methyl cellulose (CMC) L^{-1} in 24 h

15a Effects of combination treatments of R, S and GA on PDV:

Treatments	Replication 1	2	3	Average
A. Control	0	0	3.8	1.3^{a}
B. GA	6.3	5.5	3.2	5.0^{a}
C. S	47.3	36.7	17.8	33.9^{b}
D. GA + S	59.6	74.8	34.3	56.2^{bc}
E. R	1.2	0	4.2	1.8^{a}
F. R + GA	4.7	12.6	4.9	7.4^{a}
G. R + S	52.0	13.7	50.5	38.7^{bc}
H. R + S + GA	57.6	81.1	50.0	62.9^{d}
SE				10.9

15b Average effects of R, S and GA on PDV*:

Treatments	PDV	Treatments	PDV	Treatments	PDV
Without R	24.1	Without S	3.9	Without GA	18.9
With R	27.7	With S	48.0	With GA	32.9
LSD (0.005)	NS		11.5		11.5

*Main effects of R, S, and GA = 3.6 (NS), 44.1, and 14.0, respectively.

Note: The same letters in 15a denote no significant difference between treatments using Duncan's Multiple Range Test at P = 0.05.

Table 15a shows that GA also induced the production of cellulase by white clover seedlings scored by PDV in CMC solution. According to the data in Table 15b it was estimated that Rhizobium, white clover seedling, and GA contributed 6, 71, and 23%, respectively, of the total cellulase production in the culture solution.

DISCUSSION

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Field Conditions

The increases in nodule number of only 32% and top dry weight of only 28% for GA sticker over water were far less than values reported by Waggoner et al. (1979). They found that GA sticker increased plant dry weight and nodule number of tap roots by about 2 and 1.8 times that for water, respectively. Possible reasons why the measured differences between GA sticker and water treatments were not significant include the high population of the natural rhizobia, high level of the soil N, and poor experimental replication. Population of the natural *Rhizobium* could probably have out-competed the inoculant *Rhizobium*. Total soil N, which was 0.196 dag kg^{-1} (Table 16 in the Appendix), is usually considered to be "medium level." It has been well documented that increasing level of soil N reduces nodulation and N_2-fixation (Thornton, 1956; Bezdicek et al., 1974; Ratner et al., 1979). Dazzo and Brill (1978) observed a sharp reduction in the adsorption of *Rhizobium trifolli* strain 0403 to the root surface in the presence of 14 to 210 mg kg^{-1} of NO_3^--N. Experimental replication, which was

only four at the time of observation, should have been larger in order to reduce the experimental standard error.

In this experiment, inoculation did not significantly increase plant dry weight and N concentration of the plant. Nevertheless, it is still possible to calculate the benefit of inoculation in the amount of N_2 fixed. Calculation based on plant dry weight 16 weeks after planting with N concentration of the plant top at 12 weeks after planting showed that the amounts of N_2 fixed due to inoculation with water or GA sticker during 16 weeks were 19.3 and 23.6 kg N ha^{-1}, respectively. The benefit of using GA sticker was only about 4.3 kg N ha^{-1} in 16 weeks when compared to water.

Effect of Gum Arabic (GA) Sticker on Nodulation of Centrosema pubescens Under Field Conditions

This experiment did not show any evidence of the superiority of GA over water, probably because of high soil temperature at planting and for several days thereafter. Soil temperature at a depth of about 2 cm was about 30 to 34°C at noon. Most of the inoculant *Rhizobium* may have died before they were able to infect the germinating seeds. The growth of *Rhizobium* is usually optimum at 28°C (Vincent, 1970). According to Lie (1974), the optimum temperature for infection, nodule growth, and N_2-fixation of pea plants should be at 26°C. Day et al. (1978) claimed that the best growth and nodulation of cowpea were at 30 to 36°C. Nodulation was not found at 40°C.

The average nodule number plant, which ranged from 1.9 to 5.5 for the four treatments, was probably a direct effect of high soil temperature that lowered populations of the inoculant Rhizobium that could infect the germinating seeds. Root nodule observation showed that rhizobia were found in a deeper part of the soil. Nodulation was not found around the tap root.

Effect of Gum Arabic (GA) Sticker on Nodulation of White Clover Under Laboratory Conditions

Nodule number per plant for GA was about 1.6 times that for water at 6 weeks after planting. This was very close to the results of Waggoner et al. (1979), who observed that nodule number for GA was about 2 times that for water. The reduction in top dry weight of both inoculated and uninoculated plants for GA may have been due to the acidifying effect of GA around germinating seeds, resulting in the reduction of plant growth. This actually should not have occurred since the lime-pelleting material used should have neutralized the acidifying effect of GA.

No significant difference in plant dry weight between inoculated and control plants of the current experiment, regardless of the absence or presence of GA, could have been due to the medium level of soil N. Total soil N was 0.187 dag kg^{-1}.

Effect of Gum Arabic (GA) Sticker on Nodulation of Calopogonium caeruleum Under Greenhouse Conditions

Nodule number and top dry weight of Calopogonium caeruleum for GA treatment 8 weeks after planting were 2 and 2.1 times higher for GA than for water. The reduction in plant dry weight of the inoculated plants where only water was used as sticker agrees with the experimental results reported by Waggoner et al. (1979). Unlike the experiment with white clover under laboratory conditions, the current experiment showed a significant interaction between sticker and inoculation. The use of GA with lime as the pelleting material significantly increased nodule number ($P < 0.05$), nodule dry weight ($P < 0.05$), top dry weight ($P < 0.05$), root dry weight ($P < 0.05$), and N concentration of the plant tops ($P < 0.05$).

Dry weights of inoculated plants (using water as the sticker) were significantly lower than uninoculated plants, probably due to a greater consumption of carbohydrates in nodulated plants, as discussed by Gibson (1966), who also found reductions in dry weight of nodulated plants. According to Gibson (1966), such reduction only occurs at early stages of nodule initiation and development. At these stages, carbohydrate requirement cannot be measured against N_2-fixation. Waggoner et al. (1979) even found reductions in dry weight of inoculated plants at 114 and 153 d after inoculation. At 114 d after planting they found a reduction of about 40% below the control. Reductions in yield of inoculated legumes have also been reported by Subba-Rao

(1976) based on field experiments with peanut in India. The reductions in yields ranged from 0.2 to 23.5% below the control.

Concerning carbohydrate consumption in nodules, Minchin and Pate (1973) concluded that about 32% of the assimilated carbon goes to nodules. In root nodules, carbohydrates are used as substrate for plant cells and bacterial respiration. The rates of respiration or O_2 uptake usually increase at infected parts of plants (Allen, 1953; Bushnell and Allen, 1962; Shaw, 1963; Thrower, 1965), such as occur in root nodules.

Effect of Different Stickers on Nodulation of White Clover Under Laboratory Conditions

This experiment did not show a significant difference in any pair of treatments on the parameters under study, possibly due to too few replications and to too many treatment sets. Seed pelleting of each treatment was prepared one by one from treatments A to I. Treatments were placed in a refrigerator immediately after pelleting.

There was no significant difference in nodulation as affected by water or commercially used stickers at 2.5 dag L^{-1} concentration, probably due to the low concentrations of the stickers. The 2.5 dag L^{-1} concentration was chosen arbitrarily since all of the stickers tested were water soluble at that concentration.

Effect of Gum Arabic (GA) on the Growth of Rhizobium in a Defined Medium

The significant increase in Rhizobium cell numbers as GA concentration was increased from 0 to 50 g L^{-1} was probably due to an increased source of energy as GA hydrolyzed. On hydrolysis, GA yields L-arabinose, D-galactose, D-glucuronic acid, and L-rhamnose (Glicksman, 1969; Tyler et al., 1976). The pentose, L-arabinose, is a preferred carbon source for slow-growing species represented by Rhizobium japonicum (Allen and Allen, 1950; Neal and Walker, 1935). According to Neal and Walker (1935), L-arabinose is first converted to 2-keto-3-deoxy-L-arabinose. This is then cleaved by a specific aldolase to yield glycolaldehyde and pyruvate (Pedrosa and Zancan, 1974). L-arabinose is also known to be metabolized by Rhizobium meliloti (Duncan and Fraenkel, 1979). Elkan and Kuykendall (1982) included L-arabinose in the list of carbohydrates which could be used as energy sources for Rhizobium.

Effect of Gum Arabic (GA) on Nodulation of White Clover Grown in Fahraeus' Medium

The increase in nodule number as GA concentration was increased from 0 to 5 g L^{-1} ($P < 0.05$) was probably due to the increase in Rhizobium cell number in the medium in the presence of GA. This is in agreement with Subba-Rao et al. (1971) who reported that GA enhanced the number of infected root hairs and number of nodule primordia of cluster clover. However, their data appeared not to support their

conclusions since increasing GA concentrations from 0 to 2 g L^{-1} did not significantly increase the two parameters measured.

In the current experiment, at the time of planting, each of the two treatments was only inoculated with approximately 1,000 cells mL^{-1} since the preliminary study showed that when cell population was too high (for example 8 x 10^6 cells mL^{-1}), increasing GA concentrations did not significantly increase nodule number.

Effect of Gum Arabic (GA) Concentrations in Soil on Nodulation of White Clover Under Laboratory Conditions

The significant reduction in nodule number and plant dry weight at GA concentrations of 2.8 dag kg^{-1} (Table 10) was probably due to a "cementing" effect of GA on the soil surface which could reduce the rate of O_2 entering the rooting zone. At higher GA concentrations, the soil surface appeared to be more compact and harder than at lower concentrations. Another possible reason for the reduction of nodule number could be the increasing C/N-ratio of the soil at higher GA concentrations. According to Lie (1974), nodulation and N_2-fixation may be reduced at higher C/N-ratios. There was no indication that GA lowered soil pH, since pH measurements for all of the six treatments were approximately the same (data not given).

The reduction in the root lengths found in the second experiment at GA concentrations higher than 0.61 dag kg^{-1}

may have been due to the cementing effect of GA on the soil surface as discussed previously.

Effect of Rhizobium Cell Number per Seed on Nodulation of White Clover

Statistically, this experiment did not show a significant increase in nodule number per plant as cell number per seed increased from 3,000 to 180,000 using water as sticker. This agrees with Waggoner et al. (1979), who found no increase either in nodule number per plant or plant dry weight as cell number per seed increased from 600 to 3,000. Nodule number for GA-3,000 (3,000 cells per seed) also was not significantly different compared with Water-3,000 and Water-6,000. Nevertheless, nodule number for GA-3,000 was about 25% higher than for Water-3,000. This shows the superiority of GA sticker over water. At very high cell number per seed, nodule number, top dry weight, and root dry weight tended to decrease.

The very low ratio of actual cells per seed from their expected values using a 5 month old peat inoculant was probably due to the overestimated population of *Rhizobium* used in the calculation. *Rhizobium* population used in the calculation was based on the determination about 2.5 months earlier than the time the peat was used in the determination of actual cell numbers stuck onto seeds. In the second determination, where a more fresh peat inoculant was used, the difference in time from when the population was

determined and when the peat was used, was only 10 d and the age of the culture was only 1 month.

Using a fresh culture, water only stuck cell numbers of about 39% from their expected values, while GA stuck cell numbers of nearly 100% (Table 11). Therefore, if only water is used as sticker the factor of 2.5 should be considered in the calculation in order to get a more accurate estimate of the expected cell number per seed. Table 11 also shows that the average of GA/E ratio was more than 100%; this was probably due to the slight increase in the Rhizobium population during 10-d storage in the incubator at 28°C. If such increase really happened, the ratio of W/E could be lower than 39%.

Effect of Gum Arabic (GA) on Pectinase and Cellulase Production by Rhizobium and White Clover Seedlings

The percentage decrease in viscosity (PDV) in pectin (0.2 dag L^{-1}) and CMC (0.2 dag L^{-1}) solutions (Tables 12 and 13) did not clearly indicate pectinase and cellulase production by Rhizobium. The PDV values for both inoculated and uninoculated medium were about the same. These data also did not show significant increases in pectinase and cellulase activities as GA concentrations increased. Nevertheless, there was a tendency toward increasing pectinase and cellulase activities as GA concentration increased from 0 to 5 g L^{-1}.

There are two possible reasons why both pectinase and cellulase could not be detected in the medium. First, the

low levels of the two enzymes produced by Rhizobium make them difficult to detect. Second, the rich medium components may have repressed these enzymes.

Pectinase and cellulase could not be detected in pectin- and CMC- agar media. Zones of hydrolysis of the two hydrolytic enzymes around the paper disks were not clearly seen. In this experiment, the growing rhizobia were washed with distilled water before adding either hexadecyltrimethyl ammonium bromide or Congo red in pectinase and cellulase assays, respectively. Failure to detect pectinase activity in pectin medium was reported by McCoy (1932) and Smith (1952). Hankin et al. (1971), using phytopathogenic bacteria such as Erwinia and Pseudomonas, detected significant pectolytic activity on JG medium (Jayasankar and Graham, 1970) in the presence of potato extract. Hubbell et al. (1978) also claimed the presence of pectolytic enzymes in 24 strains of temperate and tropical rhizobia using qualitative and nonspecific pectin agar plate assay.

In early 1880, Beijerinck (cited by Fåhraeus and Ljunggren, 1959) failed to grow nodule bacteria on a cellulose substrate, indicating that the nodule bacteria did not produce cellulase to hydrolyze cellulose. Recent studies in this field showed that Rhizobium produced cellulase (Martinez-Molina et al., 1979; Morales, 1981). Martinez Molina et al. (1979) were able to detect cellulase and also hemicellulase activities in temperate (infective and non-infective) and tropical (infective) Rhizobium using

viscometric assays. They grew Rhizobium in the presence of cellobiose or GA as sources of carbohydrates. Morales (1981) detected significant cellulase activity on culture medium in the presence of plant root substances.

Significant pectinase activity from a medium in the presence of white clover seedlings inoculated with Rhizobium agrees with Fåhraeus and Ljunggren (1959) and Ljunggren and Fåhraeus (1959, 1961) but it does not agree with Lillich and Elkan (1968), Macmillan and Cooke (1969), Hunter and Elkan (1971), Bonish (1973), and Glenn and Dilworth (1979). Lillich and Elkan (1968) concluded that polygalacturonase has nothing to do with rhizobial infection.

Fåhraeus and Ljunggren (1959) and Dazzo and Hubbell (1982) questioned the origin of pectinase. Based on the nature of the treatments in the current experiment, which was a factorial of 2 x 2 x 2, results of the experiment showed that there were three possible origins of pectinase: GA, Rhizobium (R) and white clover seedlings (S) in the order of GA >> R > S. Gum arabic enhanced the production of pectinase by white clover seedlings and not by Rhizobium. The evidence for the presence of pectinase in GA was also found by spotting GA at 5 g L^{-1} on pectin-agar medium and incubating for 24 h. The presence of pectinases in GA agrees with Tyler et al. (1976).

The current experiment also showed that there were three possible origins of cellulase: seedlings of white clover (S), GA, and Rhizobium in the order of S >> GA > R.

Gum arabic seemed to enhance cellulase production by the plants but there was little evidence that GA enhances cellulase production by Rhizobium.

The significant pectinase and cellulase production by white clover seedlings in the presence of GA could have been due to a direct effect of GA as a carbohydrate source, resulting in rapid cell growth and development. During this rapid cell growth and development, significant amounts of pectinase and cellulase could be directly excreted into the medium outside the plant cell wall.

General Discussion

Experiments with white clover under laboratory and field conditions, and an experiment with Calopogonium caeruleum under greenhouse conditions to test the superiority of GA sticker over water, showed that GA sticker significantly enhanced nodulation. An experiment with Calopogonium caeruleum even showed that GA also increased nodule dry weight, plant dry weight, and N concentration of the plant tops. Evidence of enhancement of nodulation by GA was also found in an experiment where GA was dissolved in Fåhraeus' medium and in experiments where GA was directly incorporated into the soil at increasing concentrations.

Results of experiments to find the reasons how GA may enhance nodulation are summarized as follows: 1) it stuck more Rhizobium cells on seeds, 2) GA increased cell numbers of Rhizobium, 3) GA contained occluded pectinase, and 4) GA

enhanced the production of pectinase and cellulase by the plants.

In this study, the enhancement effect of GA sticker could not be separated from the effect of lime pelleting material itself, which constitutes about 500 g kg^{-1} of original seed weight. Pelleting material could possibly have reduced water losses from seeds so that the viability of rhizobia on the seed surface could have increased, especially before the seeds were exposed to water in soil. Very rapid death of rhizobia on the seed surface occurs shortly after loss of visible moisture, about 15 minutes after the seeds are inoculated (Salema et al., 1982). Bushby and Marshall (1977) reported that drying and rehydration caused injury to rhizobia and resulted in the leakage of important cell constituents. In the presence of water in soil, lime pelleting material will fall from seed and this could result in a slight increase in pH around germinating seeds. The increase in pH, especially in acid to neutral soils, will enhance the growth of rhizobia and the germinating seeds. Calcium as a cation can stimulate the growth of rhizobia in the rhizosphere (Broughton, 1978) and it can also enhance bacterial infection (Munns, 1970; Blevins et al., 1977). Calcium can also increase the uptake rate of K by roots (Epstein, 1961).

The first step that usually occurs before rhizobia infect root hairs is an increase in the Rhizobium cell numbers due to the presence of abundant organic substances

released by the roots (Alexander, 1977). Gum arabic coating of seeds in soil could probably become a source of carbohydrates to increase of Rhizobium cell numbers. The current study showed that increasing GA concentrations increased cell numbers of Rhizobium. The more rhizobia present around root hairs the greater the possibility of root infection by rhizobia. After rhizobia adhere to the root hairs, according to Dazzo and Hubbell (1982), the root hair cell wall is hydrolyzed at a highly localized site of adhesion of the infecting bacteria. Possible hydrolytic enzymes which are involved in the hydrolysis of root hair cell wall are pectinase or polygalacturonase (Fåhraeus and Ljunggren, 1959; Ljunggren and Fåhraeus, 1961; Hubbell et al. 1978), cellulase (Morales, 1981) and hemicellulase (Martinez-Molina et al., 1979). Among those hydrolytic enzymes, pectinase is believed to be the most important, since destruction of pectins in the middle lamella will cause dissolution of the cell wall and allow the cellulose microfibrils to slide freely past one another (see special topics in Salisbury and Ross, 1978). The presence of GA around germinating seeds could enhance the production of both pectinase and cellulase by the plants. This, undoubtedly, could enhance bacterial infection. Subba-Rao et al. (1971) observed increasing numbers of infected root hairs of cluster clover as GA concentration was increased from 0 to 0.2 g L^{-1}. Concerning the role of pectinase in relation to bacterial infection,

Ljunggren and Fåhraeus (1961) concluded that polygalacturanase (pectinase) plays a role in the "weakening" of root hair cell walls and this facilitates bacterial invasion.

What are the functions of hydrolytic enzymes in the plant cell itself? It is believed that the cell wall is a "giant" molecule and keeps a cell from bursting. Plant cell wall is comprised of cellulose microfibrils in a matrix of pectin, hemicelluloses, proteins, and other substances (Northcote, 1972). During cell growth, the wall molecules need to be "cut" to permit insertion of new cell wall material, and to be joined again. Hydrolytic enzymes such as cellulase, hemicellulase, and pectinase would be necessary for breaking down the existing cell wall.

This study showed the superiority of GA over water (normal inoculation) on nodulation in legumes with the following conditions:

(1) Fresh peat culture with high rhizobial population was used. The rate of peat inoculant used was 2.3 g kg^{-1} of white clover seed and 0.75 g kg^{-1} of Calopogonium caeruleum seed to give about 3,000 cells per seed, respectively.

(2) Sticker used was GA solution at 45 dag L^{-1} concentration. The rate of GA solution was 333 ml kg^{-1} of seed either for white clover or Calopogonium caeruleum or about 150 g GA kg^{-1} of seed.

(3) Pelleting material used was lime ($CaCO_3$) (300 mesh) at the rate of 513 g kg^{-1} of white clover seed and 250 g kg^{-1} of Calopogonium caeruleum seed.

(4) Seed planting was done within the time period of several minutes to less than 2 h after pelleting. Under field conditions, the white clover seeds were buried at a depth of about 1.5 cm.

(5) Watering was done immediately after seed planting and during plant growth as required.

The main advantage of using GA sticker with lime as pelleting material (Figs. 9 to 12), was that the seed pelleting method used inoculant at a rate far less than in other inoculation methods. For example, for white clover, 50 g of high quality peat inoculant should be enough to inoculate about 21.7 kg seed or for about 2.78 ha planting area, and for Calopogonium caeruleum it should be enough to inoculate about 66.67 kg seed or for about 5.55 ha planting area. If farmers are recommended to choose the seed pelleting method in legume inoculation, in addition to high quality peat and GA, pelleting material such as $CaCO_3$ must also be supplied. As explained in the previous chapter, pelleting seeds is easy. Firstly, mix peat culture with GA solution. Then mix the seeds with the mixture of GA + peat culture, and finally mix pelleting material with the inoculated seeds.

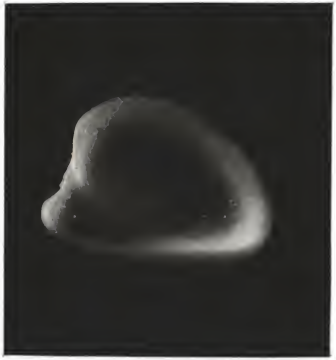

Fig. 9
White clover seed before
pelleting (50X)

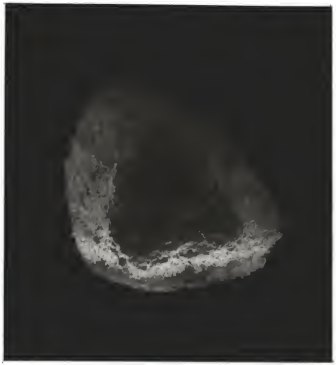

Fig. 10
White clover seed after
pelleting (50X)

Fig. 11
Calopogonium caeruleum seed
before pelleting (28X)

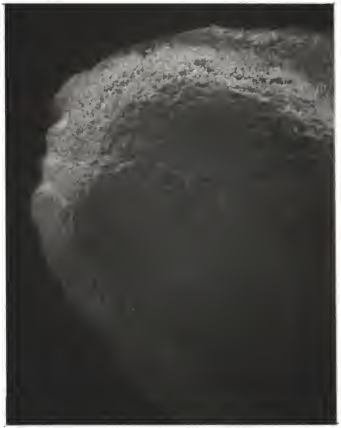

Fig. 12
Calopagonium caeruleum seed
after pelleting (28X)

Much work remains to be done in relation to the use of stickers in legume inoculation. Studies include the following:

(1) To conduct experiments on the use of stickers on a larger scale under field conditions, for example, using the following experimental treatments: (A) Uninoculated seed, (B) Uninoculated seed + lime, (C) Inoculated seed + lime with water as sticker, and (D) Pelleting seed with GA sticker and lime as pelleting material.

(2) To study the possible use of stickers other than GA and to study the possible use of pelleting materials other than lime under field conditions.

(3) To analyze the economical use of the seed pelleting method in legume inoculation based on experimental results under field conditions.

SUMMARY AND CONCLUSIONS

Five experiments were carried out to obtain experimental data as to the enhancement effect of GA on nodulation. Two experiments were conducted under field conditions, another two were under laboratory conditions, and one was under greenhouse conditions. Of the five experiments, three were carried out with *Trifolium repens* (white clover), one was with *Calopogonium caeruleum* and another one was with *Centrosema pubescens*. To explain why GA enhances nodulation, seven experiments were conducted under laboratory conditions using *Rhizobium trifolii* strain 0403. The effect of GA on increasing *Rhizobium* cell number, nodule number, plant dry weight, root length, and the production of both pectinase and cellulase was studied. The *Rhizobium* cell numbers stuck on white clover seed by water and GA as sticker were also counted immediately after the seed had been inoculated. In one of the seven experiments, the effect of GA on nodulation of white clover was studied in Fåhraeus' medium and the other one was studied in soil where GA was incorporated into the soil at increasing concentrations. The effect of GA on pectinase and cellulase was studied in two ways: (1) using an agar-plate assay method and (2) using viscosimetry.

A field experiment with white clover at 12 weeks after planting showed that nodule number per plant, using GA sticker, was 32% higher than when water was used. Under laboratory conditions, the nodule number for GA was 1.6 times the nodule number for water ($P < 0.05$). This experiment showed an inhibitory effect of GA on top dry weight of the plant. The decrease in the top dry weight was 15%, but this was not significant statistically.

A field experiment with Centrosema pubescens failed to show any significant effect of GA on nodulation. Nodule number per plant at 10 weeks after planting ranged from 1.9 to 5.5 while the nodule dry weight ranged from 7.5 to 23.3 mg per plant.

A greenhouse experiment with Calopogonium caeruleum at 8 weeks after planting showed that nodule number, nodule dry weight, top dry weight per plant, and N concentration for the plant tops for GA sticker were 2.0, 2.4, 2.1, and 1.3 times those for the water, respectively, ($P < 0.05$ for all four parameters). Top and root dry weight of the inoculated plants were about 39 and 31% lower than that of uninoculated plants where only water was used as sticker.

An experiment on the effect of different stickers on white clover nodulation, 6 weeks after planting under laboratory conditions, showed that the seven stickers tested (TIC Pretested Arabic 1 or GA, Arabic TIC Spray Dry USP Powder, TIC Pretested Karaya "B", TIC Pretested Tamarind, TIC Pretested Ghatti, Gum Arabic TIC Pretested, and Tapioca

starch) gave similar effect on nodulation. The data did not show a significant superiority of using those stickers at 2.5 dag L^{-1} as compared to water.

An experiment with a defined medium inoculated with Rhizobium trifolii strain 0403 showed that increasing GA concentrations from 0 to 50 g L^{-1} increased cell number significantly from 1.06×10^8 to 13.81×10^8 cell mL^{-1} at 7 d after inoculation. The relationship between GA concentrations (X) in dag L^{-1} and cell numbers mL^{-1} (Y) was $Y = (1.88 + 0.51 X - 0.006X^2)10^8 (R^2 = 0.88, P < 0.05)$. Maximum Rhizobium cell number was reached at a calculated GA concentration of 46.4 g L^{-1}.

An experiment with Fåhraeus' medium showed that nodulation of white clover by rhizobia grown on 5 g GA L^{-1} was 1.5 times that of rhizobia grown on 0 g GA L^{-1} ($P < 0.05$) at 15 d after inoculation.

Experiments on sandy soil under laboratory conditions, where GA was incorporated into the soil, showed that nodule number of white clover was increased as GA concentration in the soil increased up to 0.7 dag kg^{-1} but declined at higher GA concentrations. The relationship between increasing GA concentrations (X) in dag kg^{-1} in soil from 0 to 2.8 dag kg^{-1} of soil and nodule number per plant (Y) in the first experiment on sandy soil at 12 weeks after planting was $Y = 21.68 + 3.50X - 2.92X^2$ ($R^2 = 0.47$, $P < 0.05$). The calculated concentrations of GA for maximum effects on increasing nodule number, top dry weight, and root dry

weight were 0.6, 1.2, and 1.2 dag kg^{-1} of soil, respectively. In the second experiment, where GA concentrations ranged from 0 to 1 dag kg^{-1} of soil, the relationship between GA concentrations (X) in dag kg^{-1} of soil and nodule number per plant (Y) was $Y = 0.85 + 5.59X - 3.02X^2$ ($R^2 = 0.70$, $P < 0.05$). The calculated GA concentrations for maximum effects on increasing nodule number and root length were 0.93 and 0.61 dag kg^{-1}, respectively. These experiments showed that at GA concentrations of more than about 1 dag kg^{-1} of soil, nodule number and white clover growth were reduced.

An experiment on the effect of increasing Rhizobium cell number per seed of white clover did not show a significant effect on increasing nodule number and plant dry weight although the expected cell number per seed was increased 60 fold from 3,000 to 180,000. At expected cell number per seed of 3,000, the nodule number for GA was about 25% higher than for water. Determination of actual cell numbers stuck on seeds of white clover where water and GA were used as stickers showed that GA stuck Rhizobium cells on seeds 2.5 to 3.0 times that for water. Using a fresh peat inoculant, GA stuck Rhizobium cells at about 100% of the expected cell numbers while water stuck only about 39%.

Experiments on the effect of GA on pectinase and cellulase production by Rhizobium and white clover seedlings showed the following. In the absence of white clover, in a defined medium inoculated with Rhizobium trifolii strain

0403, increasing GA concentrations did not significantly increase pectinase and cellulase activities measured by the PDV of pectin and CMC solutions. There was a tendency towards increasing PDV in pectin solution (0.2 dag L^{-1}) from 6.3 to 7.6 due to increasing GA concentrations from 0 to 5 g L^{-1}. The increase in the PDV in the pectin solution could have been due to the increase in pectinase activity in the presence of 5 g GA L^{-1} in the medium. Where sterile tap water was used as culture solution, the PDV's in pectin solution (0.2 dag L^{-1}) in 24 h for the control, gum arabic (GA), white clover seedlings (S), GA + S, Rhizobium (R), R + GA, R + S, and R + S + GA were 5.3, 27.3, 3.3, 79.0, 10.4, 32.2, 13.2, and 86.4, respectively. In CMC solution (0.2 dag L^{-1}) the PDV's were 1.3, 5.0, 33.9, 56.2, 1.8, 7.4, 38.7, and 62.9, respectively. It was suggested from this experiment that Rhizobium, white clover seedlings, and GA contributed 8, 33, and 59%, respectively, of the total amount of pectinase production in the culture solution and 6, 71, and 23%, respectively, to the total amount of cellulase production.

Four of the five experiments that dealt with the use of stickers to enhance nodulation showed that GA significantly enhanced nodulation. Of the five experiments the one that failed to show a significant effect of GA on nodulation was that with Centrosema pubescens under field conditions. Nodulation was very poor in that experiment.

It was concluded from this study that GA sticker significantly enhanced nodulation. The reasons were that it stuck more Rhizobium cells onto seeds and increased cell numbers. Gum arabic contained pectinase, which could be important in "weakening" the plant cell wall, as could GA-induced pectinase and cellulase production by the plant.

In practice, the use of stickers with lime as pelleting material should be considered as a standard method of legume inoculation with Rhizobium to enhance nodulation since the amount of N_2 fixed by legumes is generally highly correlated with nodule numbers on the plant roots.

APPENDIX

Table 16

Chemical properties of soils used for laboratory, greenhouse and field experiments

	Soil for field experiments	Soil for laboratory and greenhouse experiments
pH	6.5	6.8
Total N (dag kg^{-1})	0.196	0.187
NO_3^--N (mg kg^{-1})	28	37
NH_4^+-N (mg kg^{-1})	20	16
C (dag kg^{-1})	2.52	2.48
C/N ratio	12.9	13.3
P (mg kg^{-1})	1090	1296
K (mg kg^{-1})	140	328
Mg (mg kg^{-1})	312	348
Ca (mg kg^{-1})	3600	3840

Note: pH was determined in 1:2 ratio of soil:water; total N, NO_3^--N and NH_4^+-N were determined using the procedures given by Bremner (1965); C (carbon) was determined using Walkley and Black method (Jackson, 1958). Phosphorus, K, Mg and Ca were extracted in Mehlich I solution (0.05 M HCl in 0.0125 M H_2SO_4). Potassium, Mg, and Ca were analyzed by atomic absorption spectrophotometry; Phosphorus was analyzed colorimetrically.

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I certify that I have read this study and that in my opinion conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David H. Hubbell, Chairman
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